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THE RAMAN EFFECT AND CHEMICAL BONDS IN CERTAIN ORGANIC LIQUIDS[1]

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Abstract

The paper gives a brief review of what the new mechanics has done to explain the phenomenon of incoherent scattering. An expression is derived for the frequencies characteristic of three elastically bound masses vibrating in a straight line. This simple harmonic theory is used to assist in interpreting experimental results. The value of the stretching force of all single bonds is assumed to be the same. Double and triple bonds are taken as having a stretching force twice and three times respectively that of a single bond. The value of the stretching force of a single bond is deduced with the aid of the assumption that the frequency 300 mm^{-1} arises from longitudinal oscillations of the form C—H. Spectrograms are given together with diagrams and measurements for two groups of substances: (1) ethylene glycol and five derivatives, (2) four nitriles and benzyl alcohol. Acetonitrile, phenylacetonitrile and benzyl alcohol have been studied by other authors. The rest are observed for the first time. With the aid of the simple harmonic oscillator theory previously mentioned some attempt is made to associate certain frequencies with definite bonds and structures.

Since the discovery of the Raman effect in 1928 it has been clearly seen that a new avenue of attack had been provided for the unravelling of the complexities of molecular structure in transparent substances. Very early in the history of investigations on the phenomenon certain frequencies and groups of frequencies were found to be almost precisely repeated in a number of distinctly different compounds. This fact seemed to indicate that the vibrations characteristic of certain groups of atoms persisted without regard to changes in the rest of the structure.

A number of suggestions as to the origin of particular Raman frequencies have been made by workers on the subject. Some of these, such as the assignment of frequencies in the neighborhood of 300 mm^{-1} to the C—H linkage vibrating longitudinally, are on quite a sound experimental basis; others are not quite so sound. On some there has been a certain amount of controversy. Daure originally assigned 144 mm^{-1} (approximately) to the vibration C—C. Other writers have attributed it to hydrogen, and Andrews (1) has made an interesting calculation from simple harmonic theory showing how transverse

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vibrations of hydrogen might cause just such a frequency. In a later part of this paper it will be indicated that frequencies of this order could very readily arise from a carbon chain oscillating longitudinally. Indeed, it seems that until our theoretical machinery for handling the vibrations of complicated molecules is more refined it will be difficult to say to which origin lines in this region belong, although it can be said qualitatively that both are possible.

The object of the present work is to extend and confirm such assignments of frequencies from investigations of the Raman effect of two groups of compounds. As an auxiliary aid to this, some simple vibrational theory has been used.

Theory

The general theory of the Raman effect has been well established by the new mechanics, both by wave and matrix methods. The latter is perhaps the simpler and quicker method of arriving at the final formula. $H_0^{(1)}(p_0^{(1)}, q_0^{(1)})$ is taken as the Hamiltonian of the undisturbed scattering medium and $H_0^{(2)}(p_0^{(2)}, q_0^{(2)})$ as the Hamiltonian of the disturbing field far from the scattering medium. $H_0^{(1)}$ and $H_0^{(2)}$ are as usual diagonal matrices. The momentum and space co-ordinates of the two systems $p_0^{(1)}, p_0^{(2)}, q_0^{(1)}$ and $q_0^{(2)}$ are Hermitean matrices. A resultant Hamiltonian H for the two systems coupled is then formed. This is given by

$$H(p_0^{(1)}, q_0^{(1)}, p_0^{(2)}, q_0^{(2)}) = H_0^{(1)} + H_0^{(2)} + \lambda H_1(p_0^{(1)}, q_0^{(1)}, p_0^{(2)}, q_0^{(2)}) + \cdots$$

where λ is a small constant. H is not in general a diagonal matrix, hence a transformation matrix S is sought such that W, given by the equation $SHS^{-1} = W$, is a diagonal matrix. On expanding S and W in terms of λ an expression for S can be derived from the equation $SHS^{-1} = W$ by equating coefficients of like powers of λ from the left and right members. This gives

$$S_1(n_1, n_2 : m_1, m_2) = \frac{H_1(n_1, n_2 : m_1, m_2)}{h\nu(n_1, m_1) + H_0^{(2)}(n_2) - H_0^{(2)}(m_2)}$$

where $S = 1 + \lambda S_1 \cdots$

The problem is then completely solved by suggesting a form for the function H_1 which will satisfy the fact that the disturbing force in the Raman effect is a harmonic wave. We therefore define

$$\lambda H_1 = Ee \sqrt{\frac{2\pi^2 \nu_0}{h}} \left[q_0^{(2)}(0, 1) + q_0^{(2)}(1, 0) \right] q_0^{(1)}$$

where E is the electric vector of the incident beam and the other symbols have their usual significance.

Finally we can obtain the expression for the perturbed space co-ordinate from the equation $q^{(1)} = S q_0^{(1)} S^{-1}$ and arrive at the equation representing the scattering process

$$q^{(1)} = q_0^{(1)} + \frac{Ee}{2h} \left[\frac{q_0^{(1)}(n,k)\, q_0^{(1)}(k,m)}{\nu(n,k) - \nu_0} e^{2\pi i(\nu_0 - \nu(n,m))t} + \frac{q_0^{(1)}(n,k) q_0^{(1)}(k,m)}{\nu(k,m) + \nu_0} e^{2\pi i(\nu_0 + \nu(n,m))t} \right]$$

This statement clearly shows the occurrence of the frequencies $\nu_0 \pm \nu(n, m)$ in the scattered spectrum and furthermore shows that the Raman spectrum does not necessarily correspond with the emission or absorption spectrum. The requirement that the frequency $\nu(n,m)$ appear in the Raman spectrum is that at least one of the products $q_0^{(1)}(n,k)q_0^{(1)}(k,m) \neq 0$ whereas the appearance of $\nu(n,m)$ in emission or absorption necessitates $q_0^{(1)}(n,m) \neq 0$. In short, the condition necessary for the emission of $\nu(n,m)$ in the Raman spectrum is that there be at least one other level k such that the selection principles allow transitions from the k level to both the m and n levels. The permissibility of the transition $n \longrightarrow m$ in emission or absorption does not concern the appearance of the frequency $\nu(n,m)$ in the Raman spectrum.

In a later part of this paper the formula for the fundamental frequencies of three masses connected by elastic forces in a straight line is used. It was therefore thought worth while to indicate the general method of solving such a problem for n bodies and to derive final formulae for the three body case.

Let the masses $m_1, m_2 \ldots . m_n$ be connected in a straight line by elastic forces. Let the stretching force between them be $K_1, K_2 \ldots . K_{n-1}$ dynes per cm., respectively. Now allow the masses to be displaced distances $y_1, y_2 \ldots . y_n$ respectively in the positive direction. Setting up the differential equations of motion we have

$$m_1 \frac{d^2y_1}{dt^2} = K_1(y_2 - y_1)$$

$$m_2 \frac{d^2y_2}{dt^2} = K_2(y_3 - y_2) - K_1(y_2 - y_1)$$

. . . . . .

. . . . . .

$$m_n \frac{d^2y_n}{dt^2} = -K_{n-1}(y_n - y_{n-1})$$

Suggesting a solution of the type $A_n e^{ipt}$, substituting and transposing we have

$$\left(\frac{K_1}{m_1} - p^2\right) A_1 - \frac{K_1}{m_1} A_2 = 0$$

$$-\frac{K_1}{m_2} A_1 + \left(\frac{K_1}{m_2} + \frac{K_2}{m_2} - p^2\right) A_2 - \frac{K_2}{m_2} A_3 = 0$$

. . . . . = 0

. . . . . = 0

$$-\frac{K_{n-1}}{m_n} A_{n-1} + \left(\frac{K_{n-1}}{m_n} - p^2\right) A_n = 0$$

These equations at once give a determinant for finding p. In general there is not an algebraic solution for p but numerical solutions can always be found.

For three bodies the problem is considerably simplified and we have

$$p^4 - \left[K_1 \left(\frac{1}{m_1} + \frac{1}{m_2}\right) + K_2 \left(\frac{1}{m_2} + \frac{1}{m_3}\right) \right] p^2 + K_1 K_2 \left(\frac{1}{m_1 m_2} + \frac{1}{m_2 m_3} + \frac{1}{m_1 m_3}\right) = 0$$

which gives at once

$$\omega^2_{1,2} = \frac{1}{8\pi^2}\left\{\left(\frac{K_1}{\mu_1}+\frac{K_2}{\mu_2}\right) \pm \sqrt{\left(\frac{K_1}{\mu_1}+\frac{K_2}{\mu_2}\right)^2 - 4K_1K_2\left(\frac{1}{m_1m_2}+\frac{1}{m_2m_3}+\frac{1}{m_1m_3}\right)}\right\}$$

where $\frac{1}{\mu} = \frac{1}{m_1}+\frac{1}{m_2}$, $\frac{1}{\mu_2} = \frac{1}{m_2}+\frac{1}{m_3}$ and $p = 2\pi\omega$.

Hence if the masses m_1 and m_2, and the stretching forces K_1 and K_2 are known the two frequencies $\omega_{1,2}$ can be calculated.

Experimental

Selection and Preparation of Liquids

In choosing liquids for study it was thought advisable to take groups of similar compounds. By starting out with a given compound and then replacing atoms or atomic groups by a succession of other atoms or atomic groups the origin of the various Raman lines is a very much simpler problem to solve.

The first substance studied was ethylene glycol CH_2OH-CH_2OH. One of the hydrogens was then replaced first by CH_3, then by C_2H_5, and finally by C_3H_7, thus securing the monomethyl, monoethyl and monobutyl ethers of ethylene glycol. Another variation was made by replacing first one OH group by a chlorine atom and then both, thereby obtaining ethylene chlorhydrin and ethylene dichloride, CH_2OH-CH_2Cl and CH_2Cl-CH_2Cl respectively.

For the second group studied certain nitriles were used, namely, aceto-, propio-, *n*-butyro- and phenylacetonitrile. These illustrated among other things the triple bonding $C\equiv N$ and would show the effect, if any, of increasing the length of the chain associated with it. Phenylacetonitrile contained the six carbon ring and for this reason benzyl alcohol was also studied, as the structural formula of the latter differed only from the former in the replacement of the OH group for the $C\equiv N$ group.

All these liquids were distilled rejecting the first and last third of the distilled product, and making observations only on the middle third of the distilled liquid. Ethylene glycol and ethylene chlorhydrin still retained a troublesome fluorescence after they were distilled. The Raman effect of the mono ethers was faint and, in general, the lines were quite diffuse. The latter remark also applies to ethylene glycol and ethylene chlorhydrin. Ethylene dichloride gave a good Raman spectrum with the little continuous spectrum even before distillation. Aceto- and propionitrile were much improved by distillation. No Raman spectrum of the latter could be observed before distillation. Butyronitrile gave very unsatisfactory results even after distillation, the continuous spectrum still being very heavy. The Raman spectrum of phenylacetonitrile is strong as it shows up even in the presence of considerable continuous background. Benzyl alcohol had a fairly weak Raman spectrum, but in this case continuous background was not particularly troublesome. The accurate measurement of the Raman lines is often hampered by their diffuseness and faintness due in some cases to the continuous fluorescence.

Apparatus

The spectrograph used for the observations was constructed especially for studying the Raman effect. Except for the prisms and lens the whole instrument was constructed in the workshop of the Macdonald Physics Laboratory. Two 60° glass prisms were used for dispersing the beam of light. These have working faces which are 4″ by $2\frac{1}{4}$″. For the collimator and camera two corrected Zeiss lens *f* 4.5 with foci of 30 cm. were secured. The combination gave an instrument beautifully adapted to studying the Raman effect. It has adequate light power and at the same time gives a dispersion of about 44 Å to the millimetre at 4600 Å. The complete benzene Raman spectrum can be photographed in half an hour on Wratten and Wainwright Panchromatic plates.

The Wood method of observing the scattered beam was employed. So much has already been said on this arrangement that further comment is unnecessary.

The iron arc was used for purposes of comparison in measuring the wavelengths of the Raman lines.

Discussion

Derivatives of Ethylene Glycol

The structural formulae for these substances as given by the chemist are:

CH_2OH-CH_2OH	Ethylene glycol.
$CH_2OH-CH_2-O-CH_3$	Ethylene glycol monomethyl ether.
$CH_2OH-CH_2-O-CH_2-CH_3$	Ethylene glycol monoethyl ether.
$CH_2OH-CH_2-O-CH_2-CH_2-CH_3$	Ethylene glycol monobutyl ether.
CH_2OH-CH_2Cl	Ethylene chlorhydrin.
CH_2Cl-CH_2Cl	Ethylene dichloride.

In discussing the Raman frequencies observed in the compounds studied we shall have to resort to the formula for a three body chain oscillator deduced in an earlier part of this paper. Too much must not be expected from such a

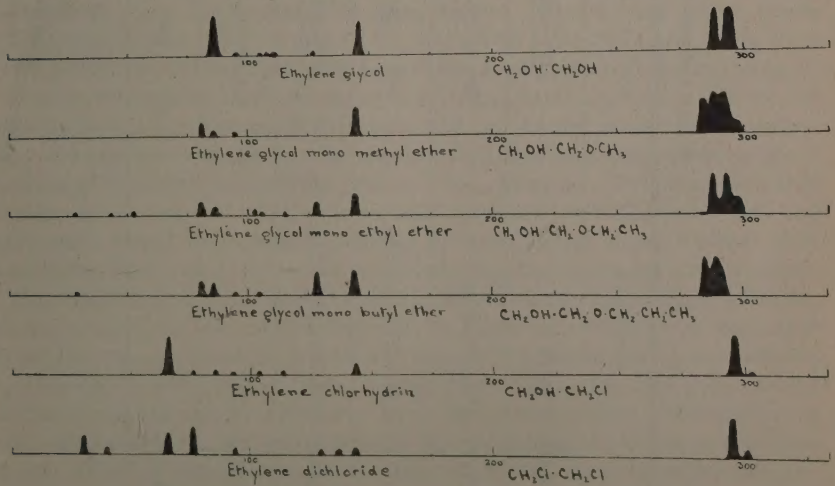


FIG. 1 *Raman effect of replacements in ethylene glycol.*

TABLE I

RAMAN FREQUENCIES (mm^{-1}) OF ETHYLENE GLYCOL AND DERIVATIVES

Compound	C–H	Vibrations due to the chain formation	Transverse vibrations
CH_2OH-CH_2OH	294 288	146 128 109 107 104 96 86	
$CH_2OH-CH_2-O-CH_3$	299(?) 294 289 283	146 95 87 83	
$CH_2OH-CH_2-O-CH_2-CH_3$	299 294 288	146 128 113 107 103 88 82 52	43 35
$CH_2OH-CH_2-O-CH_2-CH_2-CH_3$	299–290 287	146 129 113(?) 103 95 88 83	31
CH_2OH-CH_2Cl	302(?) 296	146 112 103 93 85 72 66	
CH_2Cl-CH_2Cl	301 296	143 138 130 94 72 65	40(?) 30

crude method of treating a molecular problem. Our estimate of the stretching force of a single, double or triple bond will be a rough one and care must be taken not to place any great emphasis on close numerical agreement.

Turning to the graphical representation of the frequencies for ethylene glycol and its derivatives (Fig. 1) we note that the group of lines around 300 mm^{-1} are present in all six compounds. Lines in this region are very easily excited and usually very prominent. The weight of experimental evidence has led previous writers on the subject to assign these frequencies to longitudinal vibrations between carbon and hydrogen. The fact that they are the fastest Raman frequencies observed makes it appear a very reasonable assignment. No case has been found where frequencies did not occur in this region when hydrogen was present in the compound, nor have they ever appeared in any compound not containing hydrogen. The variation from one compound to another in the numerical order of the frequency is not large, so we may assume that the vibration of this linkage is affected by neighboring linkages less than any other. This suggestion appears to be qualitatively quite reasonable. Since the assignment of frequencies near 300 mm^{-1} to longitudinal vibrations of the C−H linkage seems to be on sound experimental basis and seems relatively independent of neighboring structures, we use it in the calculations of the stretching force of a single bond by the simple formula for a two body oscillator given by

$$\nu = \frac{1}{2\pi}\sqrt{\frac{k}{\mu}} \quad \frac{1}{3.10^{10}} \quad cm^{-1}$$

where k is the stretching force of the single bond and μ is the resultant mass of the oscillator, *i.e.* $\frac{1}{\mu}$ is the sum of the reciprocals of the masses of the carbon and hydrogen atoms. This gives k a value roughly 5×10^5 dynes-cm. for calculations using the formula for three bodies. Double and triple bonds will be treated as having a value of the stretching force twice and three times respectively that of the single bond as has been done previously by Andrews (1).

These assumptions as to the identity of single bonds and the values of the multiple bonds are somewhat arbitrary and can hardly be strictly accurate. They are perhaps best justified by the fact that results of computations based on them give roughly concordant results. It is, however, obvious at once that to take satisfaction out of close numerical agreement between prediction and experiment would be extremely fatuous on this ground alone, without even considering the further fact that it is unlikely that the chains are strictly linear.

The chain structures in most of the liquids studied are of considerable length, but we have restricted our consideration of harmonic oscillators to the linear three body one, as it was not felt that at present it was worth while to make the troublesome numerical solutions of the determinant for the fundamental frequencies of the chain oscillator of more than three bodies. The manner in which similar frequencies occur, when a certain linkage is present in two compounds differing considerably, shows that to a first approximation we may sometimes, at any rate, consider a group of atoms as vibrating independently

of the remainder. This might not be so plausible in the mechanical case of masses connected by springs; however we are not dealing with such a model but with a number of dynamical systems bound together by sharing electron orbits. In such circumstances it is not at all unreasonable to assume that certain groups may, to a rough order of approximation, be treated as independent of the rest. In justification of this, in the case of the liquids here studied, we may note how very small is the modification of the frequencies 72 mm^{-1} and 66 mm^{-1} on passing from ethylene chlorhydrin to ethylene dichloride although in the transition the heavy chlorine atom has been substituted for the oxygen atom.

The formula for a three body chain oscillation has been applied to several three body chains for comparison with the ethylene glycol compounds and the expected frequencies approximately calculated.

TABLE II

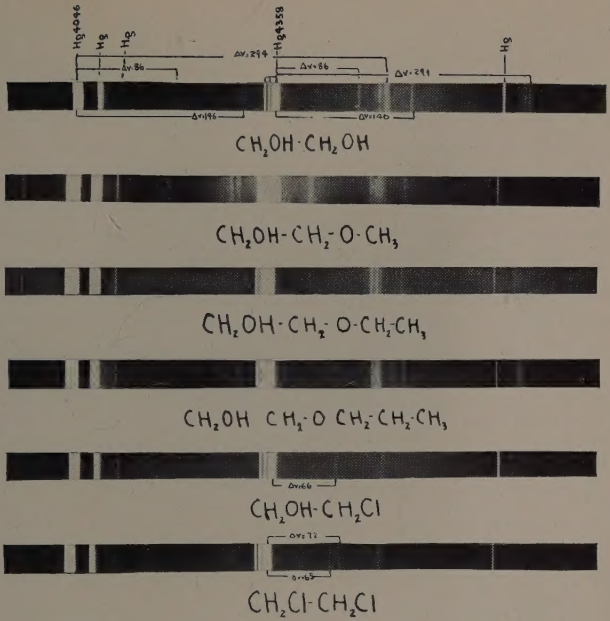
FREQUENCIES CALCULATED FROM FORMULA FOR A THREE BODY CHAIN OSCILLATION

Three body chain	Frequency mm^{-1}	
	ω_1	ω_2
C–C–C	144	84
C–C–O	143	77
C–C–Cl	140	63

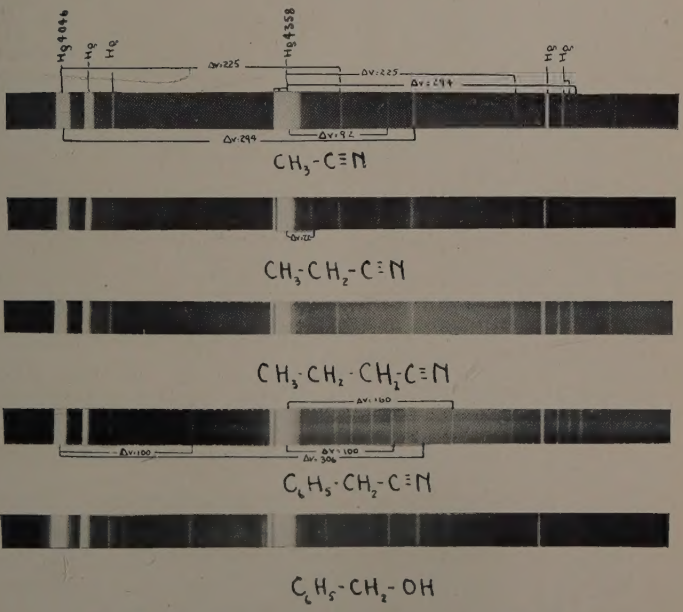
It will be noted that all these frequencies lie in a range between 50 mm^{-1} and 150 mm^{-1}. It is well known that this region is the richest in Raman lines. The lengthening of the chain will by the ordinary classical idea introduce new frequencies, but if the bonds are all still single and the masses added similar to those considered in the three body oscillation we would expect that these frequencies will still be approximately in the region 50 mm^{-1} to 150 mm^{-1}. If the chain is not strictly linear other modifications will of course be introduced, but at the present stage of development we are in ignorance as to just what the angles are between the bonds in given chains, so at the moment we must be content with using a purely linear chain. The degree of success of such treatment will be its justification.

True, this is not as definite as one would like it to be, but with premises as rough as our fundamental ones it is not justifiable to do much more than make a qualitative assignment of frequencies which at least gives us a good intuitive reason for stating that the frequencies in this region are due to a vibrating chain of atoms. It may also suggest to which atoms the stronger frequencies are due, and the effect of substituting one atom for another, if we keep our attention closely fixed on the experimental results.

With the above in mind we can now dispose of the groups of lines appearing in ethylene glycol and derivatives between 50 mm^{-1} and 150 mm^{-1} as being due to vibrations of the respective chains of atoms characteristic of each compound.



A. *Raman spectra of ethylene glycol and derivatives.*



B. *Raman spectra of certain nitriles and benzyl alcohol.*

There is more to be said however. The line at 86 mm^{-1} is very strong in CH_2OH-CH_2OH. It is present in the methyl ether, degenerated to 83 mm^{-1} and less intense. It is present and further degenerated in the ethyl and butyl ethers, and again in chlorhydrin. In the latter however it is not nearly as intense as in glycol, and only degenerated to 85 mm^{-1}. In the dichloride it is absent. The evidence seems to point to its origin as the oscillation of the oxygen atom at the end of the chain. Finally there is qualitative agreement between this assignment and the frequency 77 mm^{-1} characteristic of the oscillator C-C-O. This agreement between a three body oscillator and a four body oscillator numerically, would in general mean little but perhaps in this case, due to the particular structure of the compounds, it means more for, as was noted before, the frequencies at 72 mm^{-1} and 66 mm^{-1} are apparently repeated in the dichloride at 72 mm^{-1} and 65 mm^{-1} although there is a large difference in the mass of the substituted atom. These latter frequencies seem clearly due to chlorine, as their appearance is identical with the introduction into the compound and their intensity is much enhanced by the introduction of the second chlorine atom. It is interesting to observe how the intensity of 66 mm^{-1} is very much more intense than 72 mm^{-1} in the chlorhydrin, while in the dichloride 72 mm^{-1} is more intense than 65 mm^{-1}. This of course must be tied up with the modifications of the transition probabilites, but at the present time such molecular systems are beyond theoretical treatment.

To explain the lower Raman frequencies *i.e.*, those below about 50 mm^{-1}, it seems probable that Andrews' (1) suggestion that they are due to transverse oscillations of the chain is a good one. He showed that transverse vibrations of hydrogen might occur in the neighborhood of 140 mm^{-1}. The suggestion seems plausible on his argument but to decide on present evidence which frequencies in this region are due to such vibrations and which to the longitudinal vibrations of chains of atoms would be difficult. It is however interesting to note that Andrews' calculation of 25 mm^{-1} as the frequency characteristic of transverse vibrations of chlorine is in quite good agreement with the strong observed frequency in ethylene dichloride at 300 mm^{-1}.

It is interesting in connection with the frequencies observed in ethylene dichloride, namely, 72, 65, 40 and 30 mm^{-1}, to note that in chloroform (3) we find frequencies at 76, 67, 37 and 26 mm^{-1}. In bromoform (3) there appear similar frequencies at 66, 54, 22 and 15 mm^{-1}. The latter frequencies seem to exhibit suitable degeneration on passing from chlorine to the heavier bromine. It is therefore evident that there is good experimental evidence for saying that in ethylene dichloride the frequencies 72, 65, 40 and 30 mm^{-1} are characteristic of the presence of the chlorine atom.

The Nitriles and Benzyl Alcohol

The structural formulae of these compounds are given as

$CH_3-C\equiv N$	Acetonitrile
$CH_3-CH_2-C\equiv N$	Propionitrile
$CH_3-CH_2-CH_2-C\equiv N$	*n*-Butyronitrile
$C_6H_5-CH_2-C\equiv N$	Phenylacetonitrile.
$C_6H_5-CH_2-OH$	Benzyl alcohol.

TABLE III

RAMAN FREQUENCIES (mm^{-1}) OF FOUR NITRILES AND BENZYL ALCOHOL

Compound	C—H	C≡N	C=C	Vibrations due to the chain or ring formation	Transverse vibrations
*$CH_3-C\equiv N$	294	225		137 123 92	38
$CH_3-CH_2-C\equiv N$	300 295 290	224		147 143 131 126 108 101 84 54	37(?) 22
$CH_3-CH_2-CH_2-C\equiv N$	294 288	224		104 99(?) 83(?)	37(?)
*$C_6H_5-CH_2-C\equiv N$	306 291	224	160	119 102 100 79 61	22(?)
*$C_6H_5-CH_2-OH$	306 293		160	146 120 110 102 100 79 70 61	47 14
†C_6H_6	306 295		160 158	118 99 85 60	

*Observations have been made on these liquids by other authors (2, 4, 5).
†Results taken from Jonas Soderqvist (6).

We again find (Fig. 2) the group near 300 mm^{-1} in all the nitriles and benzyl alcohol. These frequencies are, of course, again assigned to longitudinal vibrations between carbon and hydrogen.

It is very striking to observe how the strong line at 224-225 mm^{-1} is present in all the Raman spectra of compounds in which $C \equiv N$ occurs. It is the outstanding point of difference between phenylacetonitrile and benzyl alcohol. The line is very strong in the former, and unquestionably absent in the latter. The structural formulae of the two compounds are identical save the substitution of OH for $C \equiv N$.

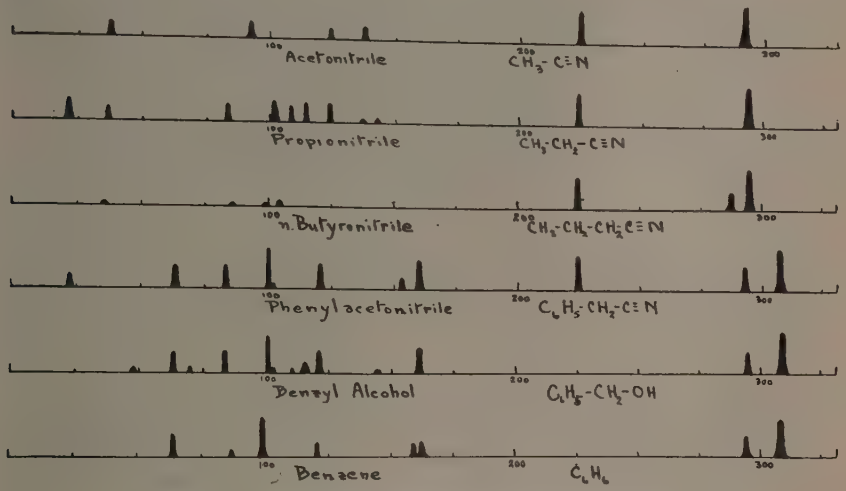


FIG. 2. *Raman effect of certain nitriles and benzyl alcohol*

In the case of acetonitrile we can apply the three body oscillator with a fair degree of assurance using $3 \times (5 \times 10^{10}$ dynes-cm.) as the stretching force of the triple bond. The two frequencies predicted are 210 mm^{-1} and 96 mm^{-1}. This is in fair agreement with the two observed frequencies 225 mm^{-1} and 92 mm^{-1} considering the approximations inherent to the process used. It is again interesting to note that the lengthening of the chain modifies only slightly the frequency 225 mm^{-1} in the higher nitriles and that the concentration of intensity in the latter compounds is still of the order of 96 mm^{-1} within 20%. It seems therefore that under the circumstances involved in these compounds the order of frequency to be expected from the oscillating chain is roughly suggested by the three body oscillator. The lengthening should, of course, introduce new frequencies, although these do not seem to appear of necessity as was shown in the case of the ethylene glycol derivatives where the lengthening of the chain in the mono ethers sometimes leads to sparser Raman effects. In the nitriles, however, on passing from aceto- to propionitrile, the complexity of the Raman spectrum increases considerably and it is very likely that butyronitrile would also give a more complicated spectrum if the troublesome fluorescence could be removed.

It is felt that the above method of explaining the origin of the frequency 225 mm^{-1} is somewhat more satisfactory than Andrews' (1) method of treating a two body oscillator in phenyl cyanide and taking the value of the elastic constant for a triple bond as four times that of a single bond. True, his numerical agreement is better, but under the circumstances, it does not seem particularly significant.

The lines between 50 mm^{-1} and 150 mm^{-1} we shall again assign to longitudinal vibrations of the chain with the possible reservation that some of the lines near 140 mm^{-1} may be due to transverse hydrogen vibrations after Andrews. The lines in this region in phenylacetonitrile and benzyl alcohol indicate how the ring structure of benzene persists and clearly shows how slight are the modifications in the frequencies of the strong lines typical of the six carbon ring. Some of the stronger lines of benzene are given in the tables and graphical representations for purposes of comparison with phenylacetonitrile and benzyl alcohol.

The frequencies near 160 mm^{-1} have been previously assigned to a double bonded carbon atom. The consistent appearance of such lines when a double bonded carbon atom is present seems to confirm this idea. It is illegitimate, of course, to suggest frequencies for the ring formation in phenylacetonitrile and benzyl alcohol from considerations of a chain oscillator. It is interesting, however, when one remembers that in other compounds not of ring form but containing a double bonded carbon atom we do obtain frequencies experimentally differing very little from 160 mm^{-1}, that an oscillator of the form $C-C=C$ gives one frequency near 180 mm^{-1} and another at about 95 mm^{-1}.

The low frequencies in the nitriles are probably due to transverse vibrations of the chain. They seem to be too small to be due to longitudinal vibrations.

Summing up the results of our discussion of these two groups of compounds we have:

(1) Frequencies in the neighborhood of 300 mm^{-1} are due to longitudinal vibrations between carbon and hydrogen.

(2) Lines near 160 mm^{-1} indicate the presence of a double bonded carbon atom in the chain or ring.

(3) Lines near 225 mm^{-1} are characteristic of the $C\equiv N$ combination in the chain.

(4) Lines between 50 mm^{-1} and 150 mm^{-1} are due to the longitudinally vibrating chain.

(5) Frequencies less than 50 mm^{-1} are probably due to transverse vibrations of the chains.

(6) Some lines near 140 mm^{-1} may be due to transverse vibrations of hydrogen.

(7) In the ethylene glycol compounds the frequency near 86 mm^{-1} was assigned to the presence of oxygen at the end of the chain, frequencies of 72 mm^{-1} and 65 mm^{-1} to longitudinal vibrations of chlorine and 42 mm^{-1} and 30 mm^{-1} to transverse vibrations of chlorine.

Acknowledgments

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THE UNITARY BEHAVIOR OF THE NERVOUS SYSTEM[1]

By Frank Allen[2]

Abstract

The investigations of the writer and his associates have shown that the responses of the organs of vision, hearing, touch, taste, temperature and pain, when stimulated, follow the same type of law, which states that some measure of the response is proportional to the logarithm of the intensity of stimulation. It has also been determined that the contraction of the muscles of the arm, the secretion of saliva, the learning process, the production of heat and the action currents in nerves, likewise conform to the same law. In every case the graphs obtained consist of two or more intersecting straight lines which indicate that, at intensities corresponding to the points of intersection, the response of the stimulated organ abruptly alters in magnitude. These changes in magnitude are due to an alteration of the balance between the reflex actions of inhibition and facilitation. Thus all the neural actions controlling the senses, muscles, glands, and probably the mental processes as well, have the same characteristic behavior.

The Weber-Fechner law has also been investigated and found to be valid in the senses of vision and taste. In the senses of learning and touch the law does not hold, but a somewhat different law was discovered that does apply.

In the long continued investigation of the varied types of responses of the stimulated organism, the muscles, glands, sensory receptors and higher centres of the intellectual and emotional faculties seem to have been studied with little reference to each other. Certain parallelisms, however, between various sensory phenomena, such as complementary and contrast effects in vision and taste, have been shown to exist; and in apparently unrelated activities, such as those of motor and visual responses, analogies have also been found.

During the last few years many of these responses in the muscles of the arm, the salivary glands, six of the senses and the learning process, have been studied by the writer and his associates with new or adaptations of older methods of measurement which permit comparison of their modes of action. The general result of these investigations has been the discovery that all types of neural activities are not merely analogous but fundamentally identical in the principles governing their responses. In this brief discussion of a very wide field of investigation the senses will first be considered, second the muscles, then the glands and finally the learning process.

It was discovered by Ferry (16), and independently later by T. C. Porter (22) who much more thoroughly investigated the phenomenon, that the critical frequency of flicker of light, *i.e.*, the frequency of interruption that was just imperceptible, was proportional to the logarithm of the intensity of the light. This is known as the Ferry-Porter law. With white light Porter found that through a very great range of intensities these quantities were represented by the equation

$$N = \frac{1}{D} = k \log I + c,$$

in which D is the duration of a flash of light on the retina at the critical frequency of stimulation, N, its reciprocal, is the critical frequency of flicker, I, the intensity of the light, and k and c are constants.

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Later Ives (17) extended the investigation to a few spectral colors with similar results, and finally the writer (5) elaborately investigated the spectrum through a much greater range of intensities than had previously been attempted. These results have shown that the Porter graphs representing the above equation, Fig. 1*, consist of from two to four rectilinear portions according to the range of intensities available in the spectrum. Other indications make it certain that an additional fifth part of the graph exists for the highest intensities of

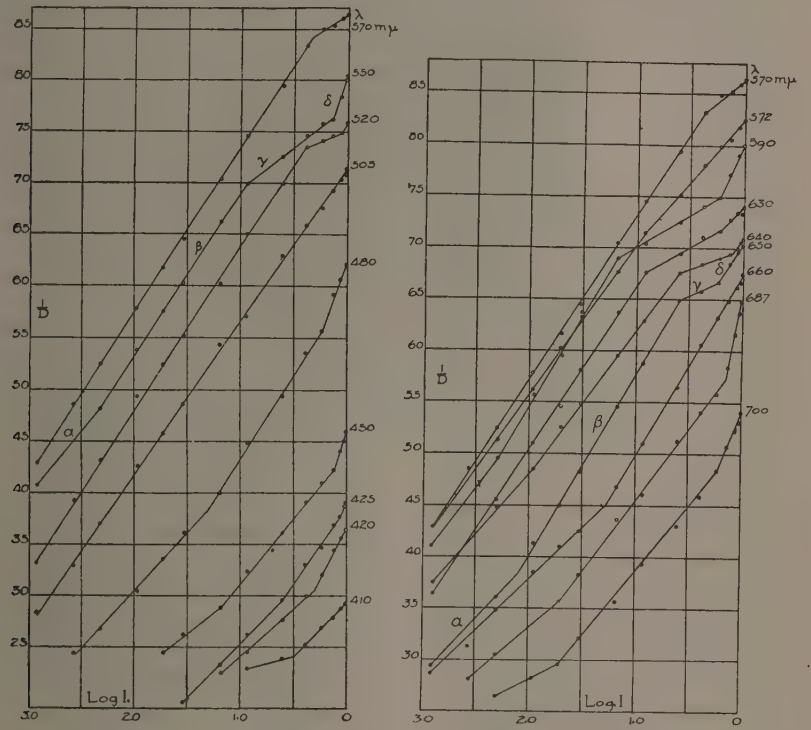

FIG. 1. *Porter graphs for spectral colors.*

stimulation. Probably if sufficient intensity could be obtained at the ends of the spectrum, the graphs for red and violet would likewise have five parts. All parts are represented by Porter's equation by changing the values of the two constants.

Beginning with the lowest range of intensities of stimulation, the successive straight lines forming a complete graph are respectively termed the α part, β part, γ, δ and ϵ parts, and the corresponding intensities of stimulation are similarly indicated. This method of designation is employed in the study of all neural responses.

It will be noticed in Fig. 1, that no two graphs are alike. This shows that each spectral color or wave frequency is far more than a mere hue or a simple

* *Fig. 1, 2, 4, 5, 7 and 11 are reproduced from the papers cited.*

component of white light; each is a stimulus with an individual physiological character. Even a very slight change in hue, for example in the graphs for the wave-lengths 0.63μ, 0.64μ, 0.65μ and 0.66μ, shows a notable physiological difference in effect. From these figures the impossibility of elucidating the intricate details of the visual processes by stimulating the retina with white light or the impure colors reflected from paper is at once apparent.

When the eye is stimulated by a single wave frequency much more than a sensation of color ensues. In addition to that conspicuous result the afferent impulses somewhere in their course release energy which, descending by the efferent paths, exercises either an enhancing (facilitation) or a depressing (inhibition) effect upon the sensitivity of the receptors themselves. It is the relative influence of these two reflex actions that differentiates the portions of the graphs from each other. The efferent impulses continue to act alternately for some time after the stimulus has been withdrawn and thus in an oscillatory manner restore to the retina its normal equilibrium.

When the retina is stimulated by any color of α intensity the inhibitory or depressing actions predominate over the enhancing, and the sensitivity of the visual receptors for all three fundamental sensations, red, green and violet, is lowered. On this account the frequent use of the eyes in dim light entails a doubly harmful effect which in time may permanently injure the visual apparatus. Hence arises the necessity of good illumination in homes, schools, factories, etc., and especially in coal mines where miners frequently suffer from a troublesome disease, called nystagmus, which is probably due in part to the low illumination.

When the stimulation of the eye is produced with colors of β intensity the three color sensations are enhanced in sensitivity, which to a greater degree is the power exercised by colors of γ intensity also. Hues of δ intensity enhance the sensations most directly stimulated and depress the remainder, while the opposite effect is produced by the brightest colors of ϵ intensity. Green of δ intensity, for example, enhances the green sensation and depresses the red and violet, but when of ϵ intensity it depresses the green sensation and enhances the red and violet.

A very large number of hitherto obscure phenomena in color vision are explained by this discovery of the inductive or reflex actions associated with vision. Two of the most important of these may be referred to here; first, the modification of the appearance of colors by simultaneous contrast, which has undoubtedly been the most difficult problem in color theory, and, second, the types of abnormal color vision.

Perception of a color invariably is formed by unequal stimulation of all three sensations in their normal states of sensitivity. When the sensations are altered in sensitivity by any means the appearance of the color must thereby be correspondingly changed. It has been shown (1) that the reflex actions induced by stimulation of the retina by any color alter the sensitivity of all receptors, but predominantly those of the sensations complementary to the hue of the stimulus, not only in the area stimulated, but also ipsilaterally in the adjacent areas, as well as contralaterally in the corresponding areas of

the unstimulated eye. A second color now impinging on one of these areas must necessarily be altered in appearance as if it were mixed with the complementary of the first color. For example, if yellow light of proper intensity falls upon a retina, the complementary blue sensation in the contiguous areas will be enhanced. A green light in addition to its normal excitation of the green sensation will therefore stimulate the blue much more than is normally possible, and green will appear bluish green. Since the innervations are reciprocal, the yellow color will also appear to be mixed with red, owing to the enhancement of that sensation by the green light; that is, it will become orange. The same explanation also holds for binocular contrast.

From the point of view of the direct and the two kinds of reflex actions excited in the visual apparatus, normal color vision can be defined not merely as the type of vision possessed by the majority of people, but as a certain delicate balance or adjustment of these actions. It is evident, therefore, that abnormal modifications in two directions are possible, which are the excessive predominance of one or other of the induced actions. These are not merely hypothetical possibilities, for definite experimental evidence of the existence of both types has now been obtained in the writer's laboratory. When the depressing or inhibitory effect is greatly in excess, color deficiency, or subnormal color vision, or color blindness, as it may variously be termed, results. This may take any one of seven forms according as the inhibition extends to any one, or two, or all three fundamental sensations. When the enhancing actions greatly predominate there may be the same number of supersensitive responses to color stimulation. Three of these types, red, green and blue anomalous trichromatic vision as it is cumbrously termed, are well known, and the remainder will probably be ultimately found.

The principle underlying the relationship of normal and the two types of abnormal visual responses may be extended to all the sensory, motor and glandular activities, and to the intellectual, emotional and volitional faculties as well. The bulk of humanity are normal in any particular set of responses; but much smaller supernormal and subnormal groups invariably are found. Thus the conclusions inferred from these discoveries in vision lead to a basic principle which in the broad fields of neurology and psychology has the widest application.

These investigations have amply confirmed the principle of trichromasy set forth in Young's theory of color sensations. By identifying reflex actions with the assimilative processes in Hering's theory of opponent colors, it becomes possible to bring the hitherto hostile essential features of the two classic theories of vision into harmonious relationship with each other.

The Sense of Taste

By intermittent stimulation of the gustatory receptors on the surface of the tongue with a periodically interrupted electric current, Miss M. Weinberg and the writer (12) found that a critical frequency of gustation occurs similar to the critical frequency of flicker in vision. With any intensity of the stimulus,

which is not the electric current itself but the quantity of electrolysed saliva, four critical values may be obtained for the duration of the stimulus. When extensive measurements with different intensities of stimulation are made they are represented by the four graphs in Fig. 2. By applying various substances such as sugar, salt, acetic acid and sulphate of quinine to the tongue and repeating the measurements, the identification of the graphs with the four fundamental sensations of taste becomes possible. Each graph consists of three linear portions which are represented by the equation

$$\frac{1}{D} = -k \log Q + C,$$

in which D is the duration of the stimulus at its critical frequency, Q is the quantity of electricity to which the electrolytic stimulus is proportional, and k and c are the usual constants. The negative sign indicates the direction of the slope of the graphs. The equation otherwise is similar to that for vision.

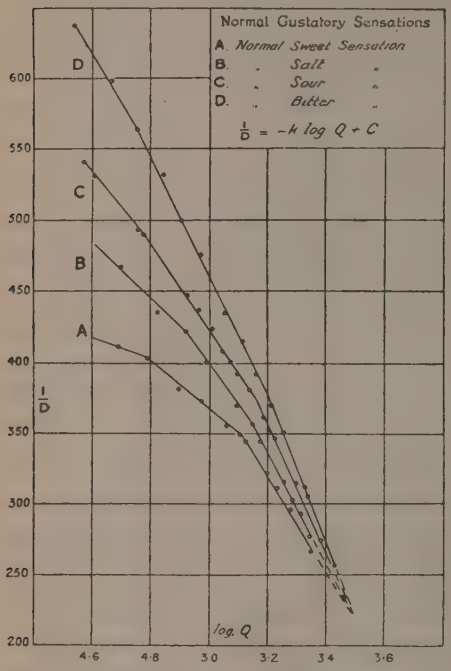

FIG. 2. *Graphs for sensations of taste.*

By stimulating the right and left sides of the tongue with solutions of the materials mentioned above, before the measurements were taken upon the right side of the tongue, the effects of both direct and reflex actions were discovered. As the sensations differ much in sensitivity, the most sensitive, bitter, being about ten thousand times more responsive than the least sensitive, sweet, the concentrations of the solutions are not physiologically comparable. Of those used in the experiments for direct action, quinine (0.125%) depressed all four sensations, sugar (20%) enhanced them all, salt (10%) and acetic acid (1.5%) enhanced the sweet sensation and depressed the remaining three. A weaker solution of sugar (5%), which was probably of α intensity, depressed the sensitivity of all four sensations.

By reflex action the same solutions of salt and acetic acid enhanced the four sensations while the quinine depressed them. The reflex action of sugar was not determined.

It is proved, therefore, that with both ipsilateral and contralateral stimulation, the processes of inhibition and enhancement operate systematically in the sense of taste in much the same way that they do in vision.

Tastelessness is not the absence of stimulation of the gustatory receptors, but the result of equality of excitation of all four sensations. It is to be

regarded as the gustatory equivalent of whiteness in vision. Should the four sensations be stimulated equally by any mixture of substances, tastelessness results. For this reason an aqueous solution of sugar and quinine of proper proportions can be prepared by which the separate tastes are apparently neutralized. But neutralization of such a character really has no meaning; the two substances simply excite the four sensations equally and so produce a sensation devoid of any particular taste. This phenomenon is equivalent to the effect of complementary colors in vision.

Since the receptors for the sweet sensation are enhanced and the remainder depressed by acids and salts, a normally tasteless substance like distilled water can no longer stimulate equally the four sensations, but necessarily excites the selectively enhanced sweet sensation predominantly, with the result that a sensation of sweetness is perceived.

The Cutaneous Sensations

The Sense of Touch

When a fine jet of air incident upon the tip of a finger is periodically interrupted by a sectored disk, Hollenberg and the writer (8) found that there is a critical frequency of percussion at which the separate impulses cease to be perceptible but blend into a continuous sensation. The values of the duration of the stimulus at its critical frequency depend upon the pressure of the air in the jet. Both quantities may easily be measured, the former by chronographic records of the speed of rotation of the disk and the latter by a mercury or water manometer. By plotting a series of corresponding measurements of these quantities, graphs are obtained like those in Fig. 3. For a single series of pressures, two series of values of the critical frequency of percussion are obtained which indicate the existence of two sensations which have been distinguished as the superficial and deep tactile sensations.

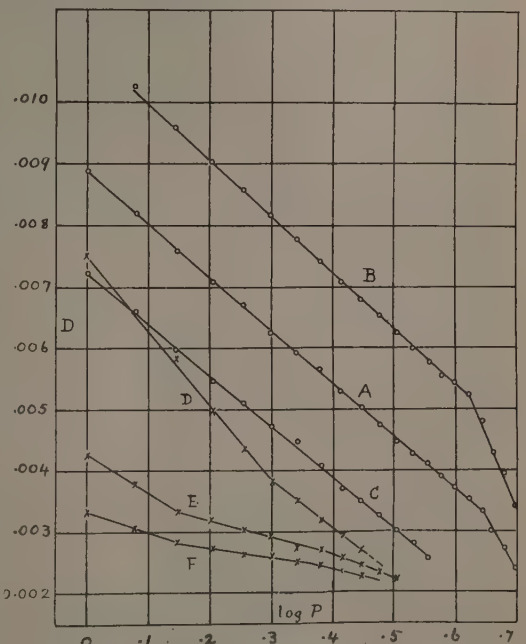

FIG. 3. *Graphs for superficial (A, B, C) and deep (D, E, F,) touch sensations.*

	Superficial	*Deep*
Normal	*A*	*E*
Enhancement	*B*	*D*
Inhibition	*C*	*F*

Each graph consists of three rectilinear parts which are designated by the letters α, β and γ. They conform to the equation

$$D = -k \log P + c$$

where P is the pressure of the air, and the other symbols have the same significance as before. The negative sign denotes the slope of the graph.

Instead of the finger tip, the lip which is more sensitive may also conveniently be employed, which has been done by Miss Weinberg in her verification of the previous work.

When the receptors are gently excited with α intensities of stimulation before measurements of the critical frequency of percussion are made, which may be done by lightly brushing the skin with a feather, it is found that both sensations become enhanced in sensitivity. Stimulation of β and γ intensities, on the contrary, has the power of depressing their sensitivity. In these respects the cutaneous receptors act in a manner opposite to the behavior of vision and probably of taste also.

Stimulation of one finger with the higher intensities causes enhancement of sensitivity in the receptors of the others. Similarly, stimulation of one area of a lip enhances the sensitivity of the adjoining areas.

The peculiar sensation of tickling is probably due to the passage of a light stimulus over the tactile receptors at a rate sufficiently slow to enable the enhancing impulses evoked to supersensitize the receptors in advance. For when the stimulus moves very rapidly no sensation of tickling is felt. Thus a critical velocity of the stimulus should be found at which this sensation vanishes, the determination of which would give the velocity of nerve conduction in the reflex arc.

If a rest period of 10 or 15 min. is taken after stimulation of a finger and then measurements of the critical frequency of percussion be repeated, the sensitivity of the finger is found to be much enhanced; while, if no rest period is allowed before repetition, the tactile sensitivity is found to be depressed. It is clear, therefore, that oscillations of sensitivity follow stimulation of receptors by which normal equilibrium is restored.

The Senses of Temperature and Pain

To some extent the senses of temperature and pain have been investigated by Macdonald and the writer (9) with a modification of the method of intermittent stimulation. The sensation of warmth was found to be so sluggish in its response that no measurements could be made with it. The sensation of cold, however, proved to be susceptible of measurement in the following way. A small metallic electrode moistened with water was secured to an area of the forearm, or the leg above the ankle, and the circuit completed by dipping the hand in water in a metal dish or by a wet bandage around the ankle.

When the current was interrupted at a high frequency no sensory effect of any kind could be discerned. But a reduction of the frequency to a certain critical value caused a sensation of cold to be suddenly perceived as if a piece of ice had touched the skin. By varying the strength of the current, the critical rate of interruption, at which coldness appeared, varied also in such a

way that the two sets of measurements conformed to the same type of equation found in touch,

$$D = -k \log I + c$$

where I is the current strength, and the other symbols have the usual significance. As readings could not be repeated on different days with the same receptors, and only a very few could be obtained on a single area of the skin on account of the deleterious action of the current, two groups of short graphs were obtained, one for the arm and the other for the leg, the latter of which is shown in Fig. 4. Two of the graphs are sufficiently long to have two parts, while the remainder possess but one.

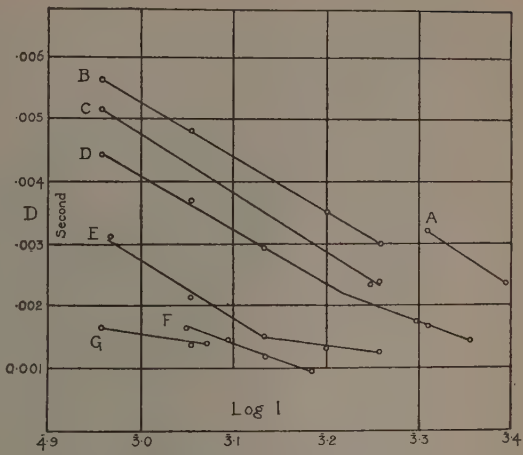

FIG. 4. *Graphs for sensation of cold in right leg.*

The Sense of Pain

The sensation of pain was investigated on both hand and leg, in the latter case by the same arrangement of electrodes as before. On the hand, the cuticle was pulled away from the base of the nail on one finger leaving only a very thin layer of skin. By placing this finger in water and a wet bandage on the wrist, a pain sensation resembling the sting of an insect was produced at a critical frequency of interruption of the current.

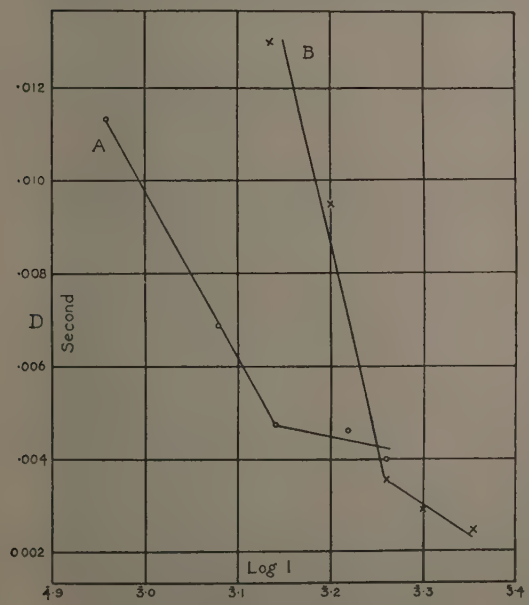

FIG. 5. *Graphs for pain sensation. A, left hand; B, right leg.*

Two graphs for measurements of the sensation of pain in the arm and leg are shown in Fig. 5, both having two linear parts which conform to the above equation.

The difficulties attendant upon these measurements prevented any attempt to obtain readings for the depressed and enhanced conditions of the sensation.

The Sensation of Hearing

The source of musical sounds employed by Miss M. Weinberg

and the writer (11) in this investigation was a tonvariator which was capable of giving a series of pure tones covering the octave from 150 to 300 D.V. This was placed in a fairly effective sound-absorbing box with a hole in one side to permit a ray of sound to emerge. In front of this hole an aluminum disk with four symmetrically placed holes in it was rotated by an electric motor in order to produce the changes in intensity necessary for intermittent stimulation of the ear.

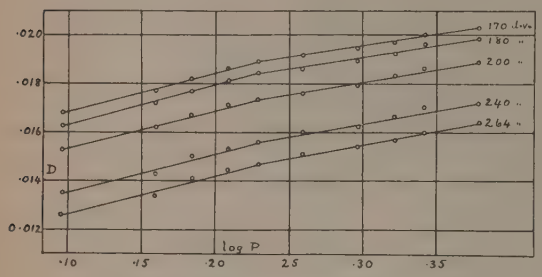

FIG. 6. *Graphs for sense of hearing.*

To obtain sufficiently pronounced effects for measurements in the extremely difficult problem of interrupting the sound, the size of the hole had to be adjusted to the wavelength, the disk covered with velvet and placed so that its pile was in contact with the box. The last adjustment was essential to success. For higher octaves even this method of control completely failed, while for the one mentioned the ideal alternation of sound and silence was by no means realized.

By varying the blowing pressure of the tonvariator and suitably changing the pitch of the tone, a group of graphs (shown in Fig. 6) was obtained, representing measurements of the critical frequency of pulsation or flutter of the tone.

Each graph consists of two linear parts which are represented by the equation

$$D = k \log P + c$$

where P is the blowing pressure of the air actuating the tonvariator, to which the intensity of the sound is proportional, D is the duration of a single tone impulse at its critical frequency of flutter, and k and c are the usual constants.

Probably if sufficiently intense tones were to be produced the graphs would be found to possess one or more additional parts as in other sensory measurements. By subjecting the ear to continuous stimulation by tones of suitable pitch from another tonvariator before measurements were made, depression of sensitivity of selective groups of the auditory receptors was produced. No measurements of enhanced sensitivity were obtained by any means then at our disposal. At that time the occurrence of post-stimulation enhancement as in touch, or the oscillatory recovery of receptor equilibrium, had not been discovered. Recently, however, J. F. Allen (14) has found the occurrence of contralateral inhibition by stimulating one ear with a musical tone and then measuring the reduction of sensitivity in the other. He has also found that post-stimulation enhancement of aural sensitivity results from both ipsilateral and contralateral stimulation. Since each stimulating tone depresses its own group of receptors, the separate effects of a number of tones acting simultaneously can be determined, thus giving a new method of tonal analysis.

The Response of Muscles

If one stands near a wall and strongly presses the back of the fingers of one hand outwards against it for about 10 sec., the arm when hanging freely will rise without effort and with a peculiar feeling of lightness. By taking advantage of this phenomenon of post-contraction, the response of the arm to stimulation was investigated by O'Donoghue and the writer (10).

Instead of pressing with unknown force against the wall, weights were hung on a wire turned into a horizontal position over a pulley and attached to a suitable stirrup in which the fingers could be comfortably placed. By lifting the weight a little above the floor for 10 sec. the arm received a measured stimulation. After releasing the stirrup it became elevated by the post-contraction of the muscles through a definite angle.

A normal series of such measurements is plotted in Fig. 7, graph *B*. The graph consists of four linear parts which conform to the equation

$$A = k \log W + c$$

where A is not a time measurement but the angle of rise, W is the weight used as a stimulus, and k and c are the usual constants. By repeating a series of measurements immediately after the first set was taken, a second graph, *C*, was obtained which showed diminished angles of elevation of the arm. This represents the partly inhibited responsiveness of the muscles. When, however, the arm was allowed to rest for 20 min. after the first series was completed and the measurements then repeated, graph *A* was obtained which shows a condition of enhanced responsiveness. Similar contralateral depressed and augmented responses of the right arm were also obtained, the first immediately after stimulating the left arm, and the second by waiting for 20 min. after stimulation. Thus the effects of ipsilateral and contralateral stimulation can be obtained in the muscles of the arms, similar to those which occur in the senses.

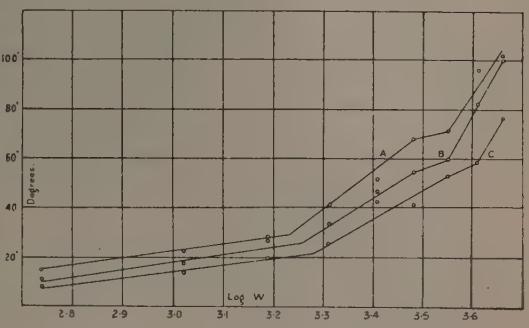

FIG. 7. *Graphs for post-contraction of muscles. B, normal; A, augmentation or enhanced responsiveness; C, inhibition.*

Since in about three minutes after stimulation of the arm, a diminished responsiveness is induced, and an augmented response 20 min. after stimulation, there must be a rest interval between these two values at which the response is normal. This was found to be a period of 10 min. Thus the normal responsiveness of the muscles is restored by an oscillation of the inhibiting and enhancing reflex actions.

Glandular Secretion

By using a method devised by Lashley for measuring the saliva secreted by the parotid gland after stimulation of the tongue, the writer (6) has found this activity of the organism to follow the same logarithmic law obtained in the senses and muscles. For this purpose a graduated series of solutions of acetic acid was used to stimulate the tongue, and for each stimulation period of three minutes the quantity of saliva secreted was measured. The results when plotted, Fig. 8, form a graph consisting of four or five rectilinear parts which conform to the equation,

$$W = k \log S + c$$

where W is the weight of saliva secreted and S is the strength of the acid stimulus.

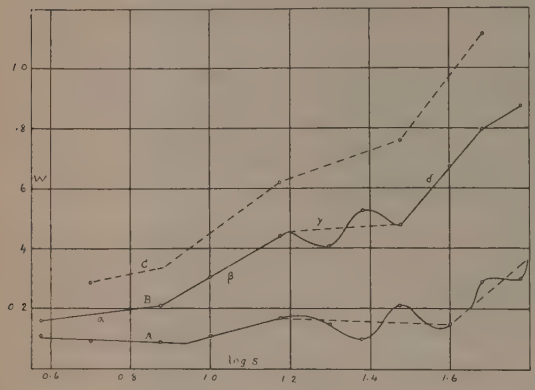

FIG. 8. *Graphs for secretion of saliva. A, stimulation for periods of 1 min.; B, for periods of 3 min.; C, enhancement after 20 min. rest.*

In the γ portion, the graph shows a marked oscillatory effect, which in some other series of measurements extends to all four portions.

Repetition of a series of measurements immediately after the conclusion of the first gave no evidence of continued depression of responsiveness but only indications of a fairly rapid oscillation of the neural forces of inhibition and facilitation.

By allowing a rest period of 20 min. after the measurements for the normal graph B were completed, an augmented response of the gland was obtained which is shown in the same figure, graph C. Graph A represents the responses for shorter stimulation periods of one minute.

Ipsilateral and contralateral stimulation of the tongue afforded some evidence that oscillations of inhibition and enhancement may occur in both halves of the neural apparatus quite independently of each other. These experiments introduce an important new principle into neurology, that bilateral oscillations of the reflex actions may take place either in the same phase or in opposite phases which may reinforce, or interfere with, each other. Probably such oscillatory effects can be elicited, under proper conditions of stimulation, in the muscles and senses also.

In vision they seem to be the cause of binocular rivalry and fusion of colors. If, for example, a red color be viewed by one eye and green simultaneously by the other, at one time red is perceived to the exclusion of green, and a little later green to the exclusion of red. Sometimes the two sensations fuse into their compound product yellow. In the first case it is possible for the inhibitory

phase to be predominant in the eye viewing the green color and the enhancing phase in that stimulated by the red. When both phases change to the opposite actions, red will be inhibited and green perceived. Should the enhancement processes be in the ascendant in both eyes simultaneously, the colors will fuse into yellow.

Motor, Electric and Thermal Effects of Stimulation

Comparable with the effects which have just been presented are some measurements taken by Sherrington (23) to show the relation between the intensity of the stimulus and the magnitude of the corresponding reflex action. The particular reflex observed was the flexion-reflex of the hind limb of a spinal dog. The stimulus consisted of a definite number of electric shocks which were varied in intensity but not in frequency. By plotting logarithms of the numbers representing intensities of the stimulus as abscissae and corresponding measures of the reflex effect as ordinates, the graph in Fig. 9 is obtained which is represented by the equation

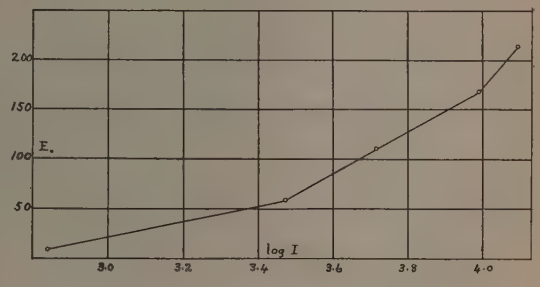

FIG. 9. *Flexion reflex for limb of dog.*

$$E = k \log I + c$$

where E is the measure of the reflex, and I the intensity of the stimulus.

The graph has three linear portions, the middle one of which is determined by three accurately placed points. The other two, however, have not sufficient points to establish their character fully.

Several other sets of observations in this work also give similar graphs, though the number of measurements, while ample for the purpose for which they were obtained, are not always sufficient to determine fully the character of each part of the graph. It is interesting to observe that the same law of neural action governs responses in the dog and man.

In the third edition of Evans' work, (15) there is a discussion of some of the work of Hill and his associates on nerve conduction, in the course of which are reproduced some measurements of the heat produced during the passage of nerve impulses. In addition there is also given in graphical form the intensity of the action currents and associated heat production.

By plotting the measurements of these various responses as ordinates against logarithms of the frequency of stimulation in electric shocks per second, the graphs in Fig. 10 are obtained. The numbers for the ordinates are the same for all graphs but their meaning differs for each. In the case of the graphs A and B, for the former of which they are multiplied by 10, they represent absolute values of the heat per impulse in 10^{-8} cal. per gm. of nerve. For C and D

the ordinates appear to be an arbitrary scale whose values are made to coincide for the stimulus value 100.

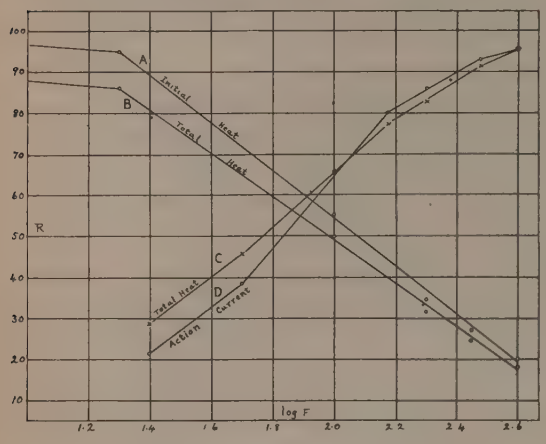

FIG. 10. *Graphs for nerve conduction.*

The first points on *A* and *B* lie too far to the left to be included in the figure, but they fall on the upper lines when produced. The remaining points clearly lie on straight lines, the slight variations being due either to errors of observation or to the oscillatory effect. The graphs *C* and *D* consist of four linear parts.

Both sets of graphs conform to the equation,

$$R = \pm k \log F + c$$

where R represents the particular form of response, and F the frequency of the electric shocks. In graphs *A* and *B* the negative sign is necessary and the positive in *C* and *D*.

The Learning Process

Up to this point we have been considering purely physiological processes associated in the case of the senses with the formation of sensations. Since the graphical results are of so general a character it seemed reasonable to suppose that psychological processes, which are necessarily associated with neural structures, would also conform to the same type of law.

Many measurements of the learning process have been made by various methods; but a group of learning curves for the acquisition of skill in typewriting, which were obtained by Book for several students and published in "The Psychology of Skill", seemed, as he had plotted them, to possess a logarithmic character. The ordinates of his curves were numbers of typewriter strokes for each practice period of half an hour, and the abscissae were numbers of days of practice.

By replotting (7) the curves with logarithms of numbers of days, the rectilinear graphs in Fig. 11 were obtained. They are represented by the equation

$$N = k \log D + c$$

where N is the number of typewriter strokes and D the days of practice.

The occasional occurrence of nearly horizontal parts of the graph, which psychologists term plateaus, has caused prolonged discussion. Its practical significance is the cessation of the power of learning during the length of such intervals. It is now evident that the same reflex processes of facilitation and inhibition, which occur in other activities of the organism, control the learning process also. The plateaus are therefore due to an inhibiting process which

depresses the responsiveness of the cortical areas concerned with learning. Probably the same phenomena occur in all intellectual, emotional and volitional faculties of the mind, a wide generalization which, while at present resting on insufficient experimental evidence, has many indications of the occurrence of these phenomena to support it.

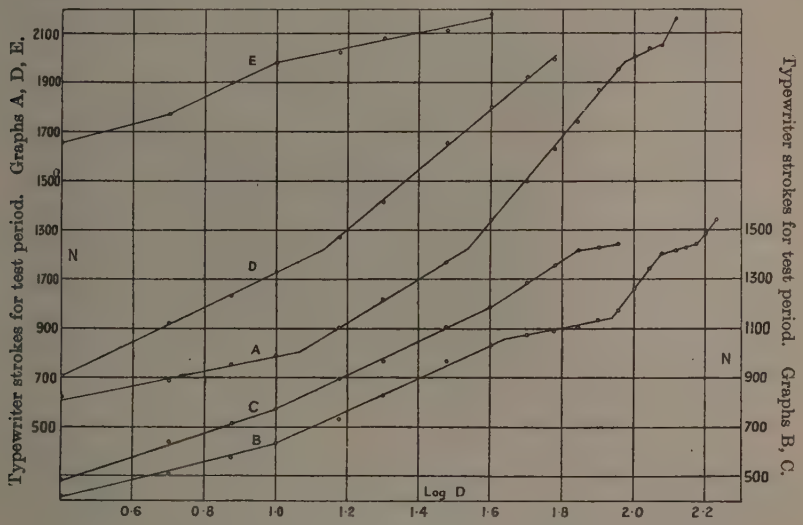

FIG. 11. *Graphs for learning process for different individuals. Abscissae are logarithms of days practice.*

Evidently recess and vacation periods in schools and universities, as well as rest periods in industry, are necessary because of the presence of oscillations of the neural reactions of both short and long periods. The times of their introduction and their duration can be determined only by experimental trials in each occupation. An intelligent application of the principles here set forth should result in an increased and improved output of mental and physical work with a simultaneous extension of the comfort of those concerned and a greater conservation of their energy.

The normal learning process consists, in part at least, of a certain balance being maintained between stimulation, enhancement and inhibition. A supernormal development of the enhancing process is probably associated with great ability and genius at one end of the scale, and at the other the excessive and permanent predominance of the inhibitory process is responsible for the subnormal mind.

Fechner's and Weber's Laws

The numerous equations which we have discussed are identical in form with Fechner's equation,

$$S = k \log I + c$$

from which they differ only by Fechner's interpretation of S as the number of hypothetical units of sensation, of which none are yet recognized.

Fechner's law is derived from Weber's law which expresses the fact that when an organ is excited by any intensity of stimulus, I, the ratio of the just perceptible increment, ΔI, to the whole intensity is always constant. This famous law has suffered during the century of its existence from the fact that all attempts to verify it experimentally have shown it to be apparently incorrect for extreme values, and only roughly approximate for a medium range. König's (18) measurements for vision, which have been regarded as the most accurate yet obtained, were made by stimulating two adjacent areas of a retina by colors of the same hue and intensity and then slowly increasing the brightness of one of them until it was just noticeably brighter than the other. That is, he inferred the action of an increased brightness of light on a single retinal area by experiments on two areas. As we now know that stimulated retinal areas mutually influence the sensitivity of each other, König's method is fundamentally defective.

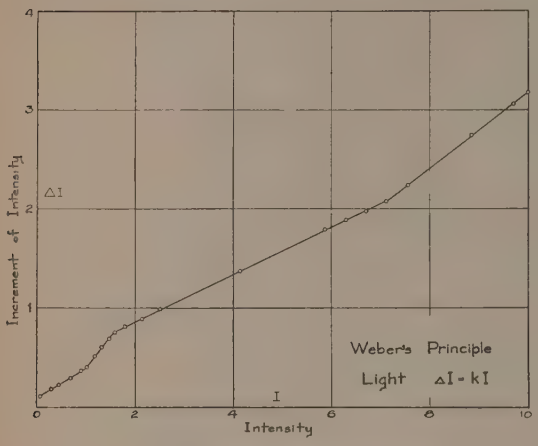

FIG. 12. *Weber's principle for light.*

During the last year two research students in the writer's laboratory, Macdonald and J. F. Allen (19) have attacked this problem with complete success. By means of the writer's tri-color spectrometer (3) a red spectral color, 0.687 μ, was thrown upon the centre of the retina. With a second collimator and a camera shutter a just perceptible flash of identical color was momentarily superposed on the first. The measurements were carried through a wide range of intensities from threshold to fairly high values. The results are shown in Fig. 12, in which the ordinates are values of ΔI, and the abscissae of I. The graph consists of four linear portions for

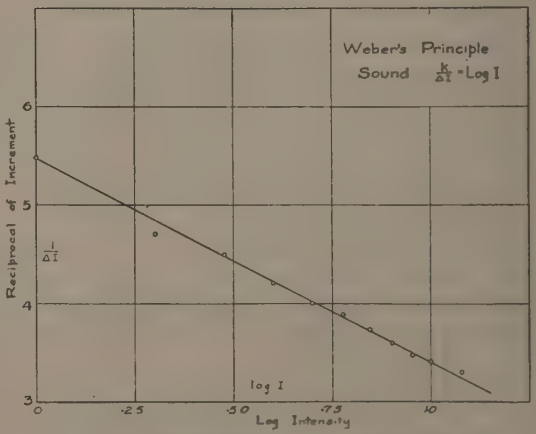

FIG. 13. *Modified Weber's principle for sound.*

each of which the Weber ratio $\frac{\Delta I}{I}$ has a different but constant value. The law, which may be written $\Delta I = kI$, is therefore correct to a high degree of precision throughout the range of intensities investigated.

By an analogous method Macdonald and Allen (20) have found the Weber law to be incorrect for sound, but they have discovered a law that does hold as the graph in Fig. 13 shows. In this case the ordinates are reciprocals of ΔI, and the abscissae values of log I. The constant ratio is thus between these two quantities, and may be written in the form, $\frac{1}{\Delta I} = k \log I$.

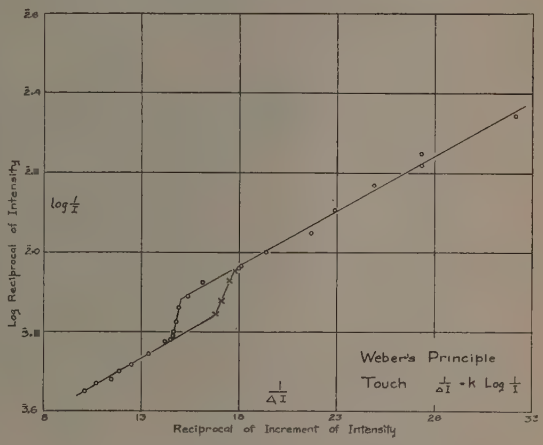

FIG. 14. *Modified Weber's principle for touch.*

Macdonald and Robertson (21) have also applied the same method to the sense of touch by using as a stimulus a jet of air under pressure. The same law that obtains in sound was found to hold in this sense also as a reference to the graph in Fig. 14 shows. For the available range of sound intensities the graph has only a single part, while the more extended range of the tactile stimulus gives four parts. In order to obtain uniformity of slope with the graphs for light and sound, the Weber principle in this case may be written, $\frac{1}{\Delta I} = k \log \frac{1}{I}$, since $\log \frac{1}{I} = -\log I$.

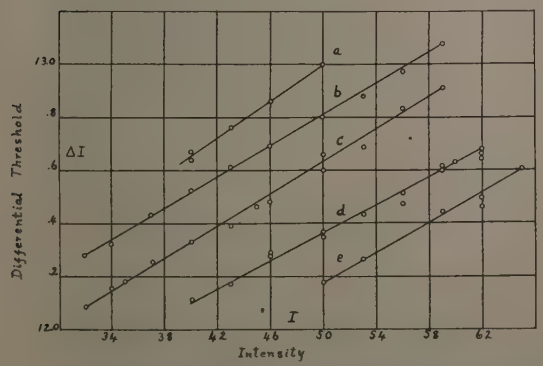

FIG. 15. *Weber's Law. Sensation of taste. Graphs b and d were identified as taste sensations. The remaining graphs are other sensations excited in the tongue.*

Weber's law applies to taste, Fig. 15, as well as to vision since in both senses the stimulation is ultimately chemical in its nature, whereas in sound and touch the stimulation is mechanical. The graphs for taste consist of but a single linear portion probably because the range of intensities of stimulation was not very great.

Conclusion

The evidence which has now been set forth may be generalized in the statement that all responses of the organism to stimulation, sensory, motor, secretory electrical, thermal and mental, follow the same laws of action. In every case the response either increases or decreases in proportion to the logarithm of the stimulus. When enhancement is in the ascendant it is denoted by the positive sign in the equation, while the predominance of the inhibitory process occasions the negative sign. The equation represents the relation between stimulus and response, but the fundamental physiological significance is concealed in the constants.

The universal occurrence of the logarithmic function in neural activities is probably due to the fact that multitudes of receptors, nerves, muscle fibres, cortical cells or impulses in a single nerve, are always involved and not individuals. In physics the same function, for example, appears in the kinetic theory of gases where again multitudes of discrete quantities are involved.

The writer has formulated the theory that reflex action characterizes the responses of the sensory apparatus as it does those of the motor and secretory complexes. It seems improbable that in the senses such effects are wholly central, while in the muscles and glands they are peripheral. The evidence we have presented, therefore, seems conclusively to demonstrate the fundamental unitary behavior of the neural structures and processes underlying the highly diversified character of all responses of the organism to stimulation. Modifications of reflex arcs are doubtless possible without changing their nature, and on this account the slightly different forms of the equations symbolizing their actions occur.

Müller's law of the specific energy of the senses, or, since energy has now a different physical meaning, of specific perceptual response to stimulation, may be completely generalized by extending its scope to the efferent nervous system also. For central stimulation of the efferent nerves causes a motor or secretory response, and an alteration of receptor, or, in the learning process, of cortical sensitivity, which is independent of the nature of the nerves or of their impulses but depends entirely upon the constitution of the tissue in which they terminate.

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STUDIES ON LIGNIN AND RELATED COMPOUNDS
V. ACTION OF HALOGENS ON LIGNIN AND WOOD[1]

By Harold Hibbert[2] and Charles A. Sankey[3]

Abstract

The addition of bromine to lignin residues and compounds of related constitution has been studied in some detail. It has been found that this addition is not only a function of time, concentration, and bromine solvent, but also of the acidity of the reaction medium. Evidence has been obtained that renders it unlikely that di-isoeugenol and di-isosafrol are derivatives of cyclobutane.

Introduction

In the preceding communications (18, 19, 20, see also 15) new methods have been described for the extraction of lignin in what appears to be a relatively unchanged condition. The evidence submitted indicates that the solvents used (glycol, glycol monomethyl ether, glycerol chlorhydrin) apparently react with the lignin and remain in combination with it, although the exact mode of union remains unknown.

This type of lignin (for example, "glycol-lignin", prepared by using glycol as the extraction medium) appears to offer many advantages as a starting material for an investigation of the structure of lignin. On the one hand, judging from the mode of formation, it would seem that no deep-seated changes could have taken place during its preparation from spruce meal and, on the other, its ready solubility in aqueous acetone and alcohol, as well as in certain anhydrous organic solvents, opens up the possibility for various new lines of attack.

Previous Work on the Structure of Lignin

Earlier investigators have, in general, confined their attention to the lignin product obtained by the action of either 72% sulphuric acid (König and Rump (25)) or 42% hydrochloric acid (Willstätter and Zechmeister (33) on wood meal. Obviously the use of such reagents is open to grave suspicion with respect to a possible deep-seated action occurring during the isolation of the lignin.

Recently Freudenberg and coworkers (6-14, 28, 30) have modified the Willstätter and Zechmeister process (33) in that they avoid the use of highly concentrated acid by substituting a treatment of alternate extractions with hot 1% sulphuric acid and cuprammonium hydroxide solution respectively.

A review of the previous literature on lignin appears to indicate that the following facts have been established:

[1] *Manuscript received February 4, 1931.*

Contribution from the Department of Industrial and Cellulose Chemistry, McGill University, Montreal, Canada, with financial assistance from the National Research Council of Canada, and the Canadian Pulp and Paper Association. Constructed from a thesis submitted by Charles A. Sankey, May, 1928, in partial fulfilment of the requirements for the degree of Master of Science at McGill University, Montreal.

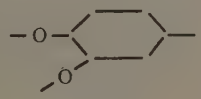
[2] *Professor of Industrial and Cellulose Chemistry, McGill University.*

[3] *Postgraduate student, McGill University.*

(a) Lignin, as present in wood, does not appear, from the limited information available, to react with halogens in the absence of moisture. In the presence of water, substitution takes place, the amount of halide acid formed being equivalent to the amount of halogen combining with the lignin (3). This would seem to indicate that the molecule, under these conditions, does not contain a readily reactive, unsaturated ethylene linkage.

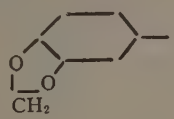
(b) Lignin contains methoxyl groups apparently present, at least in part, attached to an aromatic nucleus.

(c) The high yield of protocatechuic acid, 20%, obtained by fusion of lignin with alkali (27) supplies supporting evidence for the presence of an aromatic unit of the type A.



A

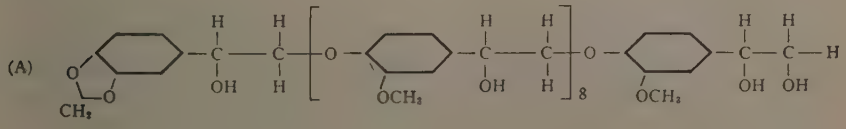
(d) The liberation of formaldehyde on treatment of lignin with dilute acid, as first pointed out by Freudenberg and Harder (11), may indicate the presence of the oxymethylene grouping B.

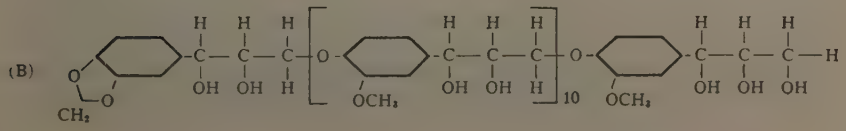


B

There would seem to be no convincing evidence either for the assumption that lignin represents a polymerized form of coniferyl aldehyde as maintained for so long by Klason (24), or of the actual presence of a carbonyl group.

In a recent paper by Freudenberg, Zocher and Dürr (14, see also 12), one of a series of researches on lignin carried out by Freudenberg and coworkers (6-14, 28, 30), the conclusion is drawn that lignin possesses either the structure (A) or (B):





Scope of Present and Proposed Future Investigations

It seems desirable that the problem of the structure of lignin should be attacked, not only from the synthetic organic, but also from the physico-chemical standpoint, and in the succeeding communications the results of some new methods of approach are to be recorded.

In the first place it is necessary to define the lines of enquiry which would seem to be of primary importance. These are as follows:

1. Does lignin contain a reactive, unsaturated ethylene linkage and what is the nature of its behavior towards halogens?

2. Is lignin present in wood as an aromatic, hydroaromatic, or aliphatic type, or as a mixture of two or more of these types?

3. What evidence is there as to the presence of either an active, or an inactive, carbonyl group in lignin? What is the mechanism of the reaction of lignin with sulphurous acid and bisulphites?

4. In addition to the methoxyl groups what other radicles or groupings are present in the lignin aggregate?

5. Is lignin present in wood merely as a type of colloidal adsorption compound, or is it present in chemical union with the pentosans, hexosans, and cellulose, either singly or collectively?

It is intended to discuss these facts in detail in later researches.

Unsaturated Character of Lignin

A few preliminary experiments were carried out some three years ago in which carefully dried spruce meal was treated with a solution of dry chlorine in dry carbon tetrachloride.

The evidence obtained at that time indicated that no reaction took place at ordinary temperature. In presence of traces of moisture a slight reaction occurred which, however, was accompanied by the formation of an approximately equivalent amount of hydrochloric acid.

From these preliminary results it might be concluded that lignin does not contain a readily reactive, unsaturated, ethylene linkage and that the action of halogen only takes place in presence of moisture and is in the nature of a substitution reaction.

In view of these results the possibility arose as to whether the action of halogen dissolved in dry carbon tetrachloride, on spruce meal, might not be complicated by two factors: (a) the solvent used for dissolving the halogen might not be suitable for the purpose; and (b) the effect of halogen on the "isolated lignin" might be different from that found in the case of spruce meal.

An extended investigation was therefore made on the action of halogens on various simpler unsaturated derivatives; on glycol lignin, and on spruce meal, using a variety of solvents for the halogen in the hope that a procedure might be developed suitable for determining the unsaturation of wood complexes and residues, particularly those lignin fractions and derivatives being studied in this laboratory. In this research the addition of bromine has been shown to

be unsuitable for the determination of unsaturation except from a purely empirical standpoint, but some interesting data have been obtained.*

Some ten bromine reagents have been studied as regards their action on, (a) spruce meal, (b) various lignin products obtained in this laboratory, and (c) unsaturated derivatives of known constitution and representing various stages of conjugation of ethylene linkages.

It was found that for the wood products the amount of bromine absorbed, after allowing for substitution reactions, was not only a function of time, concentration, and of the bromine solvent, but also varied very markedly with the acidity of the medium. Thus with a given lignin product, for a given time and concentration, more bromine was added on in the strongly acid Hanus reagent (1, p. 288) than with bromine in carbon tetrachloride which was only slightly acid due to traces of hydrobromic acid formed during the reaction. But by adding successive increments of hydrobromic acid to the latter solution, the bromine adsorption could be increased to a higher figure than was obtained with the Hanus reagent. This is also in accord with the conclusions of Williams and James (31) that hydrobromic acid acts as a catalyst for bromine addition to ethylenic compounds in non-hydroxylic solvents, and that the reaction mechanism involves a partial preliminary substitution, the hydrobromic acid removed then catalyzing the main reaction of addition to the double bond.

Within comparatively narrow ranges of experimental conditions results could be checked when either a freshly prepared solution of bromine in absolute methyl alcohol (23) or a solution of bromine in carbon tetrachloride was used, and the more important analytical figures were obtained with these two reagents. It was possible with these reagents to allow for substitution. Thus for the case of bromine in methyl alcohol each ethylene linkage on saturation results in the absorption of two bromine atoms

(A) $R-CH=CH-R' + Br_2 \longrightarrow R-CHBr-CHBr-R'$

whether both add on, or whether the reaction takes place through the medium of hypobromous acid

$$MeOH + Br_2 \longrightarrow MeBr + HOBr$$
$$R-CH=CH-R' + HOBr \longrightarrow R-CHBr-CHOH-R'.$$

In the case of a substitution reaction,

(B) $R-CH_2-CO-R' + Br_2 \longrightarrow R-CHBr-CO-R' + HBr$

two bromine atoms are used, one of which remains combined, the other being left as hydrobromic acid.

In both cases (*viz.*, addition and substitution) the amount of bromine entering into reaction is thus identical, so that if

w = wt. in grams of unsaturated substance taken,

a = total cc. 0.1 N bromine used in addition or substitution reactions (as in A or B)

b = cc. 0.1 N HBr liberated,

* *The unsatisfactory character of bromine titration for the determination of unsaturation has also been clearly indicated in several recent researches (2, 4, 5, 21).*

then $\frac{a-b}{w}$ = cc. 0.1 N Br corresponding to the unsaturation value of one gram substance,

and $\frac{a-b}{w} M$ = cc. 0.1 N Br corresponding to total unsaturation value per mole of unknown substance,

$\frac{a-b}{w} M \times 10^{-4}$ = number of litres of N bromine solution, or number of Br equivalents.

Since each ethylene linkage corresponds to two bromine equivalents, the number of ethylene equivalents in a substance of molecular weight M

$$= \tfrac{1}{2} \cdot \frac{a-b}{w} \cdot M \times 10^{-4} \quad \ldots\ldots \quad (x)$$

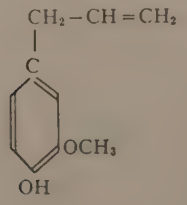
For substances of unknown molecular weight the only figure that can be calculated as a measure of unsaturation is the former value (x) divided by the gram molecular weight, this giving the number of gram double bonds per gram. Putting this quantity equal to u, then

$$u\,M = \tfrac{1}{2} \cdot \frac{a-b}{w} \cdot M \times 10^{-4}$$

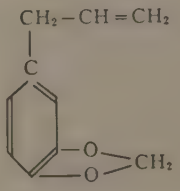
$$\text{or } u \,.\, 10^{4} = \tfrac{1}{2} \cdot \frac{a-b}{w} \cdot$$

Eliminating substitution reactions in this way and using an approximately $0.2N$ reagent at room temperature the reaction with glycol lignin (20) is practically complete in 48 hr. Using Hägglund and Urban's figures (17) of 400 for the molecular weight, unsaturation figures corresponding closely to one ethylene linkage per mole were obtained. If the acidity of the reaction mixture is increased by preliminary addition of hydrobromic acid, results up to 50% greater unsaturation have been obtained.

The compounds of known constitution studied were selected as being somewhat related to the possible lignin configuration. The marked influence of the degree of conjugation of the ethylene linkage on the reaction rate was emphasized by the study of compounds of known constitution. Thus with eugenol (I) and safrol (II)

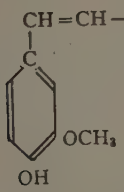


(I)

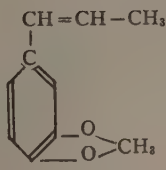


(II)

the reaction is complete (addition corresponding to one ethylene linkage per mole after making the usual substitution correction) in less than half an hour, while with isoeugenol (III) and isosafrol (IV)



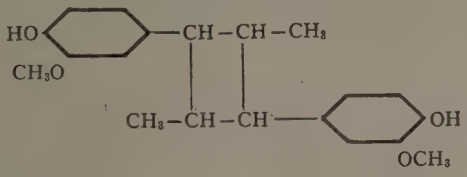
(III)



(IV)

at least four hours' reaction time was required to complete the addition.

Di-isoeugenol and di-isosafrol were also studied. The constitution of these two compounds has not been definitely established. In these cases, allowing for substitution reactions as heretofore, the bromine absorbed corresponded to two ethylene linkages per mole. On this basis either, (a) di-isoeugenol cannot be regarded as a derivative of cyclobutane (V)



(V)

the polymerization accordingly taking place through some part of the molecule other than the ethylene linkages, or through the so-called secondary valencies; or (b) the compound depolymerizes in solution under the influence of the bromine. This appears unlikely as one would expect under these circumstances that the cyclobutane ring would split in one but not in two places; that is, that analytical figures would be obtained corresponding to one and not two ethylene linkages as indicated by the experimental results.*

Brief reference may be made to the results obtained with other bromine reagents. Due to the insolubility of many of the wood constituents some reagents were not sufficiently reactive to give more than a slight action *e.g.*, the Hübl reagent (22). At the opposite extreme, Tingle's reagent, a mixture of concentrated sulphuric and hydrochloric acids (29), so completely altered the

* *It is intended to carry out a series of further investigations on the structure of these and other dimeric substances formed from products containing a conjugated linkage. (H. H.)*

products during the process of solution that no interpretation of results was possible. Still other reagents underwent rapid auto-decomposition (the bromine reacting with the solvent itself), *e.g.*, bromine in ethyl alcohol, and nascent bromine generated from equivalent quantities of bromate-bromide-acid in aqueous methyl alcohol, in aqueous ethyl alcohol or in aqueous ethylene glycol. With these latter, therefore, blank and sample analyses are not comparable over the long reaction period required with insoluble constituents. Solutions of bromine in chloroform were also tried but satisfactory check analyses were not obtained.

Experimental Part

The spruce meal used was of 100 to 200-mesh. It was extracted with benzene-alcohol mixture and dried in vacuo. The glycol-lignin was supplied by H. J. Rowley, while the eugenol, isoeugenol, safrol, and isosafrol were Kahlbaum's. The di-isoeugenol and di-isosafrol were synthesized in the laboratory by Mr. A. Paquet.* The former was prepared according to the method of Puxeddu (26) and recrystallized several times from alcohol, m.p. 181.5° C. The latter was prepared as by Glichitch (16) and recrystallized from alcohol, m.p. 145° C.

Some of the analytical results, typical of a large number of experiments, are listed in the following tables. In Table I no acid was present other than that formed in the reaction mixture, except for the Hanus reagent (1, p. 228) where the reaction medium is glacial acetic acid and in which all bromine absorbed must be assumed to be added on to ethylene linkages.

TABLE I

ANALYTICAL RESULTS OF BROMINE ABSORPTION BY SPRUCE MEAL, LIGNIN AND RELATED COMPOUNDS

Reagent	Time in hr.	$u \times 10^4$
(a) Spruce meal		
Br_2 in MeOH, approximate concentration in reaction mixture = 0.2*N*	5	5.26
	24	7.26
	44	8.14
	71	8.24
Br_2 in CCl_4, approximate concentration in reaction mixture = 0.2*N*	4	0.76
	24	1.44
	48	1.54
	72	1.50
Hanus reagent, approximate concentration in reaction mixture = 0.2*N*	0.5	1.40
	4	3.40
	24	10.40
	46	11.30
	73	12.40
	96	13.20

* *The authors desire to express to Mr. Paquet their best thanks for his kindness in this matter.*

TABLE I—*Continued*

ANALYTICAL RESULTS OF BROMINE ABSORPTION BY SPRUCE MEAL, LIGNIN AND RELATED COMPOUNDS

(b) Lignin (M=400)

Reagent	Time in hr.	No. of ethylenic linkages, $u.M$
Br_2 in MeOH, concentration as under (a)	24	1.14
	48	1.22
	48	1.18
Br_2 in CCl_4, concentration as under (a)	24	0.91
	48	1.09
Hanus reagent, concentration as under (a)	24	1.20
	48	1.50
	71	1.50

(c) Related compounds

Compound	Br_2 in MeOH		Br_2 in CCl_4		Hanus reagent	
	Time in hr.	$u.M$	Time in hr.	$u.M$	Time in hr.	$u.M$
Eugenol	1	1.28	—	—	0.5	1.00
	4	1.50	—	—	—	—
Safrol	1	1.16	—	—	0.5	0.98
	4	1.40	—	—	—	—
Isoeugenol	1	0.69	—	—	—	—
	4	1.04	—	—	40.0	1.07
Isosafrol	1	0.69	—	—	—	—
	4	0.94	—	—	40.0	0.99
Di-isoeugenol	1	1.85	4	1.97	—	—
	4	2.04	24	2.12	—	—
Di-isosafrol	1	1.60	4	1.93	—	—
	4	1.98	24	2.03	—	—

The effect of the presence of hydrobromic acid is shown in Table II. The data given is for bromine in carbon tetrachloride acting on lignin. In the case of bromine in methyl alcohol the addition of more hydrobromic acid also speeded up the rate of auto-decomposition and therefore did not give satisfactory results.

TABLE II

EFFECT OF HYDROBROMIC ACID ON ABSORPTION BY LIGNIN OF BROMINE FROM CARBON TETRACHLORIDE SOLUTION

Substance	Final acidity of reaction mixture, in cc. 0.1N acid	Time in hr.	No. of ethylenic linkages ($u.M$) (M=400)
Lignin (M 400)	0.85	24	0.91
	0.90	48	1.09
	5.0	44	1.42
	5.3	66	1.53
	10.1	48	1.55
	10.35	68	1.63

NOTE:— *Volume of reaction mixture constant.*

The figures listed in Table I and Table II are typical sample analyses from a large number of experiments.

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STUDIES ON LIGNIN AND RELATED COMPOUNDS
VI. THE MECHANISM OF AQUEOUS HALOGENATION[1]

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Abstract

The rates of addition of hypochlorous and hypobromous acids to several unsaturated compounds, under the influence of various catalysts, have been studied.

The addition of hypochlorous acid to allyl alcohol and dipropenyl glycol is catalysed by both hydrogen and chlorine ions, the catalytic effects of these ions, mole for mole, being equal. The effect of hydrogen and chlorine ions, when added together as hydrochloric acid, is not the sum of their separate effects but is proportional to their product, indicating undissociated hydrochloric acid as the real catalyst. The catalytic effect of varying amounts of added hydrochloric acid is approximately proportional to the square of the amount added, again indicating undissociated hydrochloric acid as the real catalyst.

The addition of hypobromous acid to allyl alcohol is catalysed by hydrochloric and hydrobromic acids to about the same degree. This catalysis of hypobromous acid addition by hydrochloric acid provides further support for the hypothesis of undissociated hydrochloric acid as the real catalyst in the addition of hypochlorous acid.

This catalytic effect of undissociated halogen acid on the addition of hypohalous acids is put forward as an explanation of the greater rates of addition of hypochlorous and hypobromous acids from chlorine and bromine water as compared with solutions of hypochlorous and hypobromous acids.

The addition of hypobromous acid to fumaric acid is not catalysed by hydrobromic acid but, on the other hand, is considerably decreased. It is catalysed, however, by hydrochloric acid, although not nearly to the same degree as the addition to allyl alcohol. This difference in behavior between fumaric acid and allyl alcohol shows the necessity for studying the effect of hydrochloric and hydrobromic acids on the rate of addition of hypobromous acid to a wide variety of unsaturated compounds, before any general theory of aqueous halogenation applicable to all types of unsaturated compounds can be put forward. The possible influence of conjugation has, however, been pointed out.

An attempt has been made to apply the theory of hypohalous acid addition catalysed by undissociated halogen acid to the aqueous halogenation of phenols. The reaction between hypobromous acid and *m*-nitrophenol has been shown to be catalysed by hydrochloric acid although not to the same extent as by hydrobromic acid. This indicates hypobromous acid addition, catalysed by undissociated hydrobromic acid, as one of the mechanisms involved in the aqueous bromination of phenols.

It has been pointed out that a similar mechanism cannot be the fastest one in the aqueous chlorination of phenols, since it is in disagreement with kinetic data of Soper and Smith (21), showing chlorine to react faster than hypochlorous acid with phenols.

It has been shown that resorcinol and phloroglucinol do not fall under the general classification proposed by Soper and Smith for phenols. Even here, however, the main reaction between chlorine water and the phenol seems to involve chlorine rather than hypochlorous acid.

It has been suggested that several parallel mechanisms may be involved in the aqueous halogenation of phenols and the necessity for further work in this field has been emphasized.

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Introduction

The addition of hypochlorous and hypobromous acids to unsaturated substances to give chloro- and bromohydrins is a well-known organic reaction (4, 5, 15, 16). Due to the inconvenience of preparing and using solutions of hypochlorous and hypobromous acids in large quantities, this method of preparation of halohydrins found very little application, and no interest was shown in the reaction until fairly recently, when Read and coworkers (17, 18, 19, 20), Gomberg (11) and others (2) showed that the halohydrins were formed, in some cases almost to the exclusion of the dihalides, when unsaturated compounds are treated with chlorine or bromine water*.

The effect of dilution on the relative amounts of bromohydrin and dibromide formed in the action of bromine water on some unsaturated compounds was investigated by Read and Hurst (18) and by Read and Reid (19) who found that the percentage of bromohydrin formation increased quite appreciably with the dilution. The effect of dilution is evidently due to a shift in the equilibrium $Br_2+H_2O \leftrightharpoons HOBr+HBr$, the percentage of total bromine present as hypobromous acid increasing rapidly with dilution. In fact, the change in the ratio of bromohydrin to dibromide with dilution, found by Read and Reid, is in good quantitative agreement with the values calculated for this change in ratio by allowing for the change in the ratio of hypobromous acid to bromine in the solution. The rate of formation of bromohydrin by the action of bromine water on unsaturated compounds is, therefore, evidently dependent on the concentration of free hypobromous acid, as distinguished from bromine, in the solution. This point is emphasized because it has an important theoretical significance which will be discussed later.

In none of this previous work has there been any determination of the velocity of these reactions, or any quantitative study of the effect of catalysts on the reaction velocity. Francis (10), however, in a paper on the mechanism of aqueous bromination pointed out that, while the action of bromine water on several unsaturated compounds resulted mainly in bromohydrin formation, these compounds appeared to be saturated more quickly with bromine water than with hypobromous acid solutions. He drew some theoretical conclusions from this fact which will be discussed later.

In the present investigation the rates of addition of hypochlorous and hypobromous acids to several unsaturated compounds have been determined, and the effect of catalysts on the reaction velocity has been studied. In particular, the catalytic effect of hydrogen and chlorine ions has been investigated. From the results of this work some conclusions have been drawn regarding the probable mechanism of halohydrin formation by the action of chlorine and bromine water on unsaturated compounds. These conclusions have been applied to a consideration of the mechanism of the aqueous halogenation of phenols and some preliminary experimental work has been done in this field.

* *The reaction has attained considerable industrial importance in the manufacture of chlorhydrins and glycols. It plays a marked role in the manufacture of toxic war materials such as "mustard gas" and has now been found to play an important role in the newer processes for bleaching wood pulp.*

Experimental Procedure

Materials

The hypochlorous acid solutions used were prepared by distillation of calcium hypochlorite solutions with boric acid as described by Taylor and Bostock (23). In studying the addition to dipropenyl glycol, the solutions prepared in this way were used without further purification. For the remainder of the work, they were agitated with freshly precipitated mercuric oxide to remove any traces of hydrochloric acid.

Hypobromous acid solutions were prepared by the action of aqueous bromine solution on precipitated mercuric oxide according to the method of Dancer (7), using a somewhat higher vacuum during the distillation than he used and taking advantage of the fact that the more dilute the solution, the more stable is the hypobromous acid. The solutions prepared by distillation were treated with freshly precipitated mercuric oxide before using.

Method

In most of the work twice the theoretical quantity of hypochlorous or hypobromous acid was used. Any side reactions could thus be readily detected. In the case of some of the faster reactions, only the theoretical quantity was used, the time for completion of the reaction being increased in this way. This work was originally undertaken in connection with an investigation of the action of hypochlorous acid and chlorine water on lignin, carried out at 0° C. to reduce oxidation to a minimum, and the present work was therefore also carried out at 0° C. instead of the usual temperature of 25° C. Standard solutions of all of the reactants were prepared, cooled to 0° C., mixed in the desired proportions and the reaction mixture kept at this temperature. Aliquot portions were pipetted out at regular time intervals and the unchanged hypochlorous or hypobromous acid titrated with $N/200$ sodium thiosulphate.

Some of the very fast reactions had to be measured at the end of time intervals as small as six seconds. In these cases a separate run was made for each titration, the reactants being mixed quickly and the reaction stopped at the end of the required time interval by adding acidified potassium iodide solution. The velocity constants were calculated using the bimolecular equations.

Experimental Data

Action of Hypochlorous Acid on Allyl Alcohol

During the course of the investigation over one thousand velocity constants were calculated. In order to avoid making this paper unnecessarily long, the data have been condensed as much as possible, only enough values being given to illustrate the theoretical conclusions drawn. All of the data are available in a thesis deposited in the Library at McGill University. Where only one value from a table is given, the value when the reaction is about one-tenth completed was selected, if available. In some cases, where the reaction was too fast for a measurement to be made at one-tenth completion of the reaction and direct numerical comparison of the constant with other constants was desired, the first value obtained was interpolated to one-tenth completion of the reaction by comparison with other tables.

A study of the absorption of hypochlorous acid by allyl alcohol in the presence of excess hypochlorous acid, at a concentration high enough so that the reaction is too rapid for measurement, showed that the absorption corresponds to the theoretical within the limits of experimental error and that there is no appreciable oxidation in 24 hr. The data are given in Table 1.

TABLE I

ACTION OF HYPOCHLOROUS ACID ON ALLYL ALCOHOL AT 0° C.

Time in min.	cc. *M*/20 HOCl absorbed per 25 cc. *M*/40 allyl alcohol
5	12.6
10	12.6
20	12.6
30	12.6
45	12.6
60	12.6
90	12.6
120	12.6
180	12.6
24 hr.	12.7

NOTE:—*Reactants used: HOCl, M/20; allyl alcohol, M/40. No catalyst added.*

Table II is typical of the tables of data used in compiling the condensed tables that follow.

TABLE II

ACTION OF HYPOCHLOROUS ACID ON ALLYL ALCOHOL AT 0° C.

Time in min.	cc. *M*/400 HOCl absorbed per 50 cc. *M*/1600 allyl alcohol	$k = \frac{2.303}{t(a-b)} \cdot \log_{10} \frac{b(a-x)}{a(b-x)}$
5	0.35	4.88
10	0.6	4.26
15	0.9	4.32
20	1.25	4.49
30	1.75	4.45
45	2.5	4.47
60	3.1	4.36
75	3.9	4.68
90	4.5	4.74
120	5.7	5.07

NOTE:— *Reactants used: HOCl, M/800; allyl alcohol, M/1600. No catalyst added.*

The catalytic effect of sulphuric, nitric, trichloracetic and hydrochloric acids, of sodium and potassium chlorides, and of sodium sulphate on the rate of addition of hypochlorous acid to allyl alcohol was next determined. The results are tabulated in Table III. It is seen that *M*/10000 sulphuric acid, *M*/5000 nitric acid, and *M*/5000 sodium chloride all have the same catalytic effect, that is, both hydrogen and chlorine ions catalyse the addition, and the catalytic effect of each of these ions, mole for mole, is the same. The catalytic effect of hydrochloric acid is greater than would be expected from the additive effects of hydrogen and chlorine ions of the same concentration, and is appar-

ently more nearly proportional to the product of the concentrations of these two ions than to their sum.

TABLE III

ACTION OF HYPOCHLOROUS ACID ON ALLYL ALCOHOL AT 0° C.

Catalyst	Time in min.	cc. $M/400$ HOCl absorbed per 50 cc. $M/1600$ allyl alcohol	$k = \frac{2.303}{t(a-b)} \log_{10} \frac{b(a-x)}{a(b-x)}$
None	20	1.25	4.5
$M/10000$ H_2SO_4	15	1.2	5.9
$M/5000$ H_2SO_4	15	1.7	8.6
$M/5000$ HNO_3	15	1.2	5.9
$M/5000$ NaCl	15	1.2	5.9
$M/10000$ HCl	10	1.6	12.1
$M/5000$ HCl	5	3.4	32.9
$M/2500$ HCl	5	9.9	338.0
$M/1666$ HCl	2	10.0	870.0
$M/1250$ HCl	1	9.7	1593.0

NOTE:— *Reactants used: HOCl, M/800; allyl alcohol, M/1600.*

Potassium chloride ($M/5000$) gave the same values as $M/5000$ sodium chloride; trichloracetic acid gave the same values as sulphuric or nitric acids of the same normality; sodium sulphate had no appreciable effect whatever.

If the catalytic effect of hydrochloric acid is proportional to the product of the concentrations of hydrogen and chlorine ions, it will be proportional to the square of the amount of hydrochloric acid added, so that $k = k_0 + k'x^2$ where k is the observed velocity constant, k_0 is the constant for the uncatalysed reaction, and x is the amount of hydrochloric acid added in any arbitrary units. The constancy of k' is a good test of the relationship. This relationship was tested experimentally and the values for k' for a number of experiments are given in Table IV. Since the values for k in any run increased quite appreciably with time, particularly toward the end of the reaction, the higher values were all extrapolated to one-tenth completion of the reaction by comparison with other tables. Considering the wide range of the values for k, the values for k' are as nearly constant as could possibly be expected.

TABLE IV

EFFECT OF HYDROCHLORIC ACID ON RATE OF ADDITION OF HYPOCHLOROUS ACID TO ALLYL ALCOHOL AT 0° C.

cc. $M/10$ HCl added per litre $= x$	k	k_0	$k' = \frac{k-k_0}{x^2}$
1	12.1	4.5	7.6
2	30.4	4.5	6.5
4	139.2	4.5	8.4
6	358.0	4.5	9.7
8	656.0	4.5	10.2

NOTE:— *Reactants used: HOCl, M/800; allyl alcohol, M/1600.*

Action of Hypochlorous Acid on Dipropenyl Glycol

The addition of hypochlorous acid to dipropenyl glycol and the effect of

hydrogen and chlorine ions on the rate of addition were also studied, and the same general results obtained as in the case of allyl alcohol. The effect of various concentrations of sulphuric acid and sodium chloride, both when added separately and together, is shown in Table V.

TABLE V

ACTION OF HYPOCHLOROUS ACID ON DIPROPENYL GLYCOL AT 0° C.

Catalyst	Time in min.	cc. $M/400$ HOCl absorbed per 100 cc. $M/6400$ dipropenyl glycol	k
None	5	1.0	27.5
$M/10000$ H_2SO_4	5	2.4	75.9
$M/5000$ NaCl	5	2.2	65.2
$M/20000$ H_2SO_4, $M/10000$ NaCl	5	3.5	114.0
$M/5000$ H_2SO_4	5	4.1	141.0
$M/2500$ NaCl	5	4.0	137.0
$M/10000$ H_2SO_4, $M/5000$ NaCl	5	6.8	303.0
$M/2000$ H_2SO_4	5	7.3	334.0
$M/1000$ NaCl	5	7.2	330.0
$M/4000$ H_2SO_4, $M/2000$ NaCl	1.5	8.8	1700.0
$M/1600$ NaOH	5	0.7	18.9

NOTE:—*Reactants used: HOCl, M/1600; Dipropenyl glycol, M/6400.*

Dipropenyl glycol

$$\begin{array}{l} CHOH-CH=CH-CH_3 \\ | \\ CHOH-CH=CH-CH_3 \end{array}$$

TABLE VI

ACTION OF HYPOBROMOUS ACID ON ALLYL ALCOHOL AT 0° C.

Catalyst	Time in min.	cc. $M/400$ HOBr absorbed per 200 cc. $M/3200$ allyl alcohol	$k = \frac{1}{t}\left(\frac{1}{a-x} - \frac{1}{a}\right)$*
None	0.25	7.8	5805
$M/50000$ HBr	0.25	13.3	11300
$M/50000$ HCl	0.25	11.3	10560
$M/33333$ HBr	0.1	16.0	56900
$M/33333$ HCl	0.25	16.5	24800
$M/25000$ HBr	0.133	23.4	352000
$M/25000$ HCl	0.1	17.6	76200
$M/20000$ HBr	0.1	23.9	695000
$M/20000$ HCl	0.1	20.9	163000
$M/10000$ HBr	0.1	24.1	857000
$M/10000$ HCl	0.1	23.7	584000
$M/10000$ HNO_3	0.5	13.3	7270
$M/5000$ HBr	0.1	24.6	2168000
$M/5000$ HCl	0.1	24.0	768000
$M/5000$ HNO_3	0.25	12.7	13200

NOTE:—*Reactants used: HOBr, M/3200; allyl alcohol, M/3200.*

* *This equation is employed in view of the alteration in the ratio of HOBr to allyl alcohol from 2:1 to 1:1.*

Addition of Hypobromous Acid to Allyl Alcohol

An investigation was next made of the addition of hypobromous acid to allyl alcohol and the effect of hydrobromic, hydrochloric and nitric acids on the rate of addition. The data are tabulated in Table VI. It may be seen that while

the catalytic effect of hydrobromic acid is greater than that of hydrochloric, the two are of the same order, the effect of nitric acid being much less than either.

Addition of Hypobromous Acid to Fumaric Acid

The addition of hypobromous acid to fumaric acid was studied in the same way. The results are given in Table VII. As may be seen at once, the results here are quite different. Hydrobromic and nitric acids both retard the reaction, while hydrochloric acid has a marked catalytic effect. These results will be discussed at length in the section on discussion of results.

TABLE VII

ACTION OF HYPOBROMOUS ACID ON FUMARIC ACID AT 0° C.

Catalyst	Time in min.	cc. *M*/400 HOBr absorbed per 50 cc. *M*/1600 fumaric acid	$k = \frac{2.303}{t(a-b)} \log_{10} \frac{b(a-x)}{a(b-x)}$
None	15	1.2	5.5
M/5000 HNO_3	15	1.1	5.0
M/5000 HCl	10	1.5	10.6
M/5000 HBr	15	1.2	5.5
M/1000 HNO_3	20	1.2	4.1
M/1000 HCl	5	3.7	61.1
M/1000 HBr	15	1.0	4.5
M/200 HNO_3	45	1.3	2.0
M/200 HCl	5	5.5	105.0
M/200 HBr	120	1.2	0.7

NOTE:—*Reactants used: HOBr, M/800; fumaric acid, M/1600.*

Discussion of Results

The results of the investigation of the addition of hypochlorous acid to allyl alcohol and dipropenyl glycol show that:

(1) The addition is catalysed by both hydrogen and chlorine ions and the catalytic effect of these ions, mole for mole, is the same (Tables III and V).

(2) The combined effect of hydrogen and chlorine ions, when added together as hydrochloric acid, is greater than would be expected from the separate effects of the two ions and is proportional to the product of the concentrations of the two ions rather than their sum (Tables III and V).

(3) The catalytic effect of varying amounts of added hydrochloric acid increases approximately with the square of the amount added (Table IV).

There would appear to be only two possible explanations for these results:

(A) The rate of addition may be proportional to the concentration of chlorine in the solution; that is, the mechanism of addition involves the chlorine molecule, or some other reactant which is directly proportional to the chlorine concentration at all dilutions. This explanation is consistent with the theoretical views of Francis (10) regarding the mechanism of aqueous bromination, which will, therefore, be considered at this point.

Francis (10) points out that in bromine water nascent bromine is always present, being continually formed in the dynamic equilibrium $HOBr + HBr \leftrightarrows Br_2 + H_2O$.

He considers that this nascent bromine is probably present as Br^+ and Br^- ions and that it is the positive bromine ion which is the active agent in all of the reactions of bromine water, including bromohydrin formation. In bromohydrin formation the positive bromine ion adds on to one of the carbon atoms forming the ethylene linkage, and the free positive radical thus produced then adds on a negative hydroxyl ion from the solution.

There are several objections to this point of view:

(1) The existence of positive halogen ion in chlorine and bromine water is purely hypothetical. Francis' strongest evidence for the existence of positive bromine ion, namely, the formation of mixed halogen compounds by the action of bromine water containing chlorine ion, on ethylene and fumaric acid, is now of little value because the recent work of Forbes and Fuoss (8) has shown that bromine chloride would be present under these conditions. There seems no necessity for assuming that bromine chloride adds on by ionizing to Br^+ and Cl^-, which then add on, or that positive bromine ion is involved in the formation of bromine chloride.

(2) Soper and Smith (21) have shown that in the action of hypochlorous acid on phenols, in presence of small amounts of alkali, positive chlorine ion, if formed in the equilibrium $HOCl \leftrightarrows Cl^+ + OH^-$, plays no part in the reaction, since the amount of Cl^+ ion would be inversely proportional to the concentration of hydroxyl ion, and they found that in slightly alkaline solution the reaction velocity was proportional to the concentrations of phenoxide ion and free hypochlorous acid. The only action of the alkali is its effect on the concentrations of these two reactants. In studying the effect of alkali on the rate of addition of hypochlorous acid to allyl alcohol and dipropenyl glycol (Table V), the authors have found that the apparent effect is to neutralize traces of hydrochloric acid formed in the reaction and to reduce the concentration of free hypochlorous acid. In hypochlorous acid solutions free from chlorine, then, positive chlorine ion apparently plays no part in the addition of hypochlorous acid to an ethylene linkage. This does not eliminate the possibility of positive chlorine ion playing a part in the reaction when chlorine water acts on an unsaturated compound, but there seems little reason to assume a different active agent in the two cases.

(3) It has already been pointed out that the change in the ratio of bromohydrin to dibromide with dilution, found by Read and Reid in studying the action of bromine water on some unsaturated compounds, is in good quantitative agreement with the values calculated by allowing for the change in the ratio of hypobromous acid to bromine in the solution. If both bromohydrin and dibromide formation depended on the concentration of positive bromine ion, the ratio would hardly be expected to vary in exactly this way.

(B) A second explanation, which, in the opinion of the authors of this paper, is more consistent with all of the known facts, is that the rate of addition of hypochlorous acid is proportional to the concentration of hypochlorous acid and to the product of the concentrations of hydrogen and chlorine ions; that is, the addition of hypochlorous acid may be considered to be catalysed by *undissociated* hydrochloric acid, or by some function of the product of hydrogen and

chlorine ions. In the present paper undissociated hydrochloric acid is regarded as the catalyst, but the fundamental reasoning would not be affected if a function of the product of hydrogen and chlorine ions were chosen instead.

Objection may be raised to this explanation, perhaps, in view of the very small concentration of undissociated acid present in some of the experiments. However, cases of homogeneous catalysis are known where concentrations of catalyst as low as this are effective (22, v. 2, p. 922).

It seems premature at the present time to suggest a mechanism by which undissociated hydrochloric acid catalyses the addition. A review of the data on other reactions involving addition to an ethylene linkage, however, shows that halogen acids act as catalysts under conditions where only the undissociated acid can be present. Thus Williams and James (24) found that the addition of bromine to several unsaturated compounds, in carbon tetrachloride solution, was strongly catalysed by traces of hydrobromic acid. Hydrochloric acid also catalysed the reaction, although not to the same extent.

Another reaction in which halogen hydrides act as catalysts is in the addition reaction between the halogen hydride and propylene in the liquid phase. The rate of these reactions increases enormously the instant that the molecular ratio is such that there is an excess of the halogen hydride. The best evidence of the catalytic effect was given by the reactions at $-78°$ C. where hydrogen chloride, which does not act appreciably with propylene at that temperature, catalyses the addition of the hydrogen bromide. In these reactions it was shown (14) that the undissociated halogen hydride is the active catalyst.

The rate of decomposition of hydrogen peroxide by hydrochloric acid has also been shown to be catalysed by undissociated hydrogen chloride. In this case the mechanism suggested (13) was that of an intermediate compound formation.

Another example of the catalytic action of hydrogen chloride is found in the hydrolysis of aqueous solutions of hydrogen cyanide (12). In this case it was pointed out that the increase in rate of hydrolysis corresponded to the increase in activity of the undissociated hydrogen chloride molecule.

All the above references go to show that undissociated hydrogen chloride plays a catalytic part in many reactions. Further work is being carried on by means of which it is hoped that more definite light will be thrown on the mechanism involved.

Further evidence for the catalysis of hypochlorous acid addition to unsaturated compounds by undissociated hydrochloric acid was obtained by comparing the catalytic effects of hydrochloric, hydrobromic and nitric acids on the addition of hypobromous acid to allyl alcohol (Table VI). The catalytic effect of hydrochloric acid is of the same order as that of hydrobromic acid, that of nitric acid being much less.*

The results obtained in studying the effect of hydrochloric, hydrobromic and nitric acids on the rate of addition of hypobromous acid to fumaric acid

**This evidence is weakened by the possibility of a reaction between hydrochloric acid and hypobromous acid, resulting in the formation of hydrobromic acid,* $HCl + HOBr \rightleftharpoons BrCl + H_2O \rightleftharpoons HOCl + HBr$ *although there is apparently nothing in the literature regarding such a reaction.*

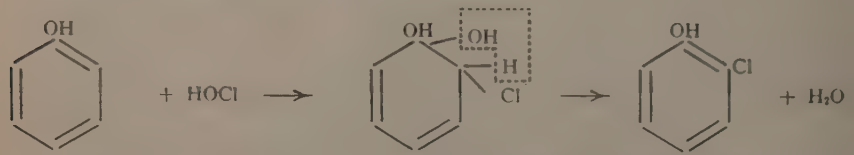
are rather unusual in that hydrochloric acid has a marked catalytic effect, while nitric and hydrobromic acids both retard the reaction. The catalytic effect of hydrochloric acid may be due to formation of bromine chloride. Nitric acid reduces the concentration of fumarate ion, thus slowing down the reaction (19). Hydrobromic acid reduces the concentrations of both the fumarate ion and free hypobromous acid. The absence of the catalytic effect of hydrobromic acid, so marked in the addition to allyl alcohol, is rather remarkable however.

The fact that the ethylene linkage in fumaric acid is conjugated with two carbonyl groups may possibly be the cause of this difference in behavior. Böeseken (3) has found that in unsaturated compounds in which the ethylene linkage is conjugated with two carbonyl groups, the ethylene linkage is not attacked by perbenzoic acid.

It is evident that not enough different types of unsaturated compounds, nor enough compounds of any one type, have been studied to enable any broad generalizations to be drawn. The addition of hypochlorous and hypobromous acids to various types of conjugated and unconjugated unsaturated compounds, and the catalytic effect of halogen and other acids on the rate of addition, should be investigated. In the meantime the tentative suggestion may be put forward that, in the case of simple unsaturated compounds where there is no conjugation, addition of hypochlorous or hypobromous acids is catalysed by undissociated halogen acids; in the case of conjugated compounds, the mechanism of addition is more complicated and there is no catalysis by undissociated acid. It must be emphasized that this is purely a suggestion. The phenols, which are to be discussed next, cannot be considered as conjugated compounds, if classified by their behavior in this respect.

Application of Hypothesis of Catalysis by Undissociated Halogen Acid to a Consideration of the Aqueous Halogenation of Phenols

The high velocity of aqueous halogenation of phenols, as compared with the velocity of halogenation in non-aqueous solutions, indicates that the hypohalous acids play a part in aqueous halogenation, the mechanism possibly involving addition of the hypohalous acid to one of the double bonds of the phenol with subsequent splitting off of water.



Considerable experimental work has been carried out in attempting to prove or disprove this theory of the hypohalous acids as the active agents in aqueous halogenation. Cofman (6) studied the iodination of phenols and obtained very good evidence that hypoiodous acid was the active agent involved. Baines (1), from a study of the effect of traces of water on the bromination of

phenols in carbon tetrachloride solution, concluded that hypobromous acid added on to the double bonds of the phenol with subsequent splitting off of water. Francis (9) opposed this view and made a direct comparison of the rates of bromination of *m*-nitrophenol when treated with bromine water and hypobromous acid solution respectively. With bromine water the reaction velocity was one thousand times as fast as with a hypobromous acid solution of the same normality. This seemed to eliminate the possibility of hypobromous acid as the active agent in the case where bromine water was used.

If, however, the catalytic effect of the undissociated hydrobromic acid in bromine water on the rate of addition of hypobromous acid to an ethylene linkage is taken into consideration, it is readily seen that Francis' results do not eliminate the possibility of hypobromous acid as the active agent in the bromination of phenols by bromine water. Quite similar results are obtained in the case of allyl alcohol (Table VI).

The hypothesis of hypobromous acid addition to the double bonds of the phenol, catalysed by undissociated hydrobromic acid, was tested out by comparing the effects of hydrobromic, hydrochloric and nitric acids on the rate of reaction between *m*-nitrophenol and hypobromous acid. The results are given in Table VIII.

TABLE VIII

EFFECT OF HYDROBROMIC, HYDROCHLORIC AND NITRIC ACIDS ON THE RATE OF REACTION BETWEEN *m*-NITROPHENOL AND HYPOBROMOUS ACID

Catalyst	$k = \frac{1}{t}\left(\frac{1}{a-x} - \frac{1}{a}\right)$
None	5000
M/10000 HBr	165000
M/10000 HCl	27200
M/10000 HNO_3	294
M/3333 HBr	271200
M/3333 HCl	53120
M/3333 HNO_3	134

NOTE:— *Reactants used: HOBr, M/3200; m-nitrophenol, M/9600.*

The retarding effect of nitric acid on the reaction is, of course, due to the depression of the ionization of the phenol. The catalytic effect of hydrochloric acid, while considerably less than that of hydrobromic acid, is still of the same order (cf. however, Maass and Sivertz (14)). Considering the fact that in the case of hydrobromic acid a considerable part of the hydrobromic and hypobromous acids react together to form bromine, thus lowering the concentration of both of these reactants, the effect of hydrobromic acid on the rate of reaction is sufficiently greater than that of hydrochloric acid to suggest that hypobromous acid addition catalysed by halogen acid is not the only reaction, or even the fastest reaction involved. In the case of phenols, bromine itself, probably nascent bromine as suggested by Francis, seems to play the main part in the reaction, although a parallel reaction involving hypobromous acid must occur.

The strongest evidence against the hypohalous acids as the main active agent in the aqueous halogenation of phenols is found in the work of Soper and Smith (21) on the reaction between hypochlorous acid and several phenols. They found the rate of chlorination of a number of phenols by $M/1000$ hypochlorous acid, in the presence of $M/5000$ hydrochloric acid, to be monomolecular and independent of the nature and concentration of the phenol. Apparently the rate measured was that of the formation of chlorine. Chlorine, therefore, must react with phenols faster than hypochlorous acid, even when the action of the latter is catalysed by hydrochloric acid.

The phenols which Soper and Smith studied (*p*-cresol, phenol and *p*-nitrophenol) have been shown by Francis' partition experiments (9) to have relatively low rates of bromination. It does not, therefore, follow that the same results would be obtained with all phenols. To test this point, the action of hypochlorous acid on resorcinol and phloroglucinol, which have very high rates of bromination, was studied. The selection of these two phenols was also influenced by the fact that the great interest in aqueous halogenation centres mainly around the enormous velocities observed with some phenols.

Soper and Smith found the rate of formation of chlorine from HCl and HOCl to be $k=2.15\times10^{-6}$ at 25° C. At a concentration of hydrochloric acid of 2×10^{-4} the reaction would be 90% completed in about 30 min. In the case of both resorcinol and phloroglucinol the main part of the reaction is completed in a much shorter time than this, even when no hydrochloric acid is added and the reaction is carried out at the lowest dilution which can be conveniently used (Tables X and XII). While a small amount of hydrochloric acid is formed in the reaction (about 25% of the hypochlorous acid absorbed), the concentration of hypochlorous acid used is low enough so that the concentration of hydrochloric acid cannot become high enough to give a rapid rate of formation of chlorine.

The rate of reaction between hypochlorous acid and these phenols is, therefore, of a much higher order than that of the reaction between hypochlorous acid and the phenols studied by Soper and Smith, and the results do not fall under their classification.

Preliminary experiments on the reaction between hypochlorous acid and resorcinol, using excess hypochlorous acid, showed that there is a fairly definite end-point to the reaction at five moles of hypochlorous acid absorbed per mole of resorcinol. About one mole of the hypochlorous acid is used up in formation of hydrochloric acid. From a study of the time required for completion of various stages of the reaction at different dilutions, it was found that there is first a very rapid bimolecular reaction in which three moles of hypochlorous acid are absorbed per mole of resorcinol, and that a slower but still quite rapid monomolecular reaction, in which two moles of hypochlorous acid per mole of resorcinol are absorbed, is either superimposed on, or follows, the bimolecular reaction. The monomolecular reaction depends on the concentration of resorcinol, not on the concentration of hypochlorous acid. The values for the monomolecular velocity constant were calculated at one dilution and are given in

Table IX. The values for the bimolecular constant were calculated for the lowest concentration and are given in Table X.

TABLE IX

ACTION OF HYPOCHLOROUS ACID ON RESORCINOL AT 0° C.

Time in min.	cc. $M/200$ $Na_2S_2O_3$ per 50 cc.	cc. $M/400$ HOCl absorbed per 50 cc. $M/5000$ resorcinol	$k = \frac{2.303}{t} \log_{10} \frac{a}{a-x}$
2	7.0	13.0	....
5	5.6	14.4	0.0744
10	3.8	16.2	0.0788
15	2.5	17.5	0.0982
20	1.7	18.3	0.0772
30	0.6	19.4	0.1041

NOTE:— *Reactants used: HOCl, M/1000; resorcinol, M/5000. No catalyst added.*

TABLE X

ACTION OF HYPOCHLOROUS ACID ON RESORCINOL AT 0° C.

Time in min.	cc. $M/400$ HOCl absorbed per 200 cc. $M/20000$ resorcinol	$k = \frac{2.303}{t(a-b)} \log_{10} \frac{b(a-x)}{a(b-x)}$	$k = \frac{2.303}{t} \log_{10} \frac{a}{a-x}$
0.1	2.3	9070	...
0.25	5.3	10600	...
0.5	8.3	11100	...
1.0	11.1	...	...
2.0	12.9	...	...
5.0	15.3	...	0.138
10.0	17.5	...	0.131
15.0	18.6	...	0.125
20.0	19.2	...	0.109

NOTE:— *Reactants used: HOCl, M/4000; resorcinol, M/20000. No catalyst added.*

It will be noticed that there is an increase in the rate of the monomolecular reaction with dilution indicating that it is affected by the degree of ionization of the resorcinol.

The effect of various concentrations of hydrochloric and nitric acids on the reaction velocity was determined and the results are given in Table XI. The results tend to support the view that, even in the case of resorcinol which reacts so rapidly with hypochlorous acid, chlorine reacts faster than hypochlorous acid.

The reaction is retarded by nitric acid due to depression of the ionization of the resorcinol. Hydrochloric acid at a concentration of 10^{-4} has the same retarding action as nitric acid at the same concentration, but hydrochloric acid at a concentration of 10^{-3} catalyses the reaction. Calculation shows that with hydrochloric acid at a concentration of 10^{-4}, the rate of formation of chlorine is much slower than the uncatalysed rate of reaction between hypochlorous acid and resorcinol. Chlorine cannot, therefore, be formed fast enough to play any part in the reaction. With hydrochloric acid at a concentration of 10^{-3} the rate of formation of chlorine is greater than the uncatalysed

rate of reaction between hypochlorous acid and resorcinol. The increase in the observed rate in this case, therefore, would seem to depend on the intermediate formation of chlorine.

TABLE XI

ACTION OF HYPOCHLOROUS ACID ON RESORCINOL AT 0° C.

Catalyst	Concentration of catalyst	Time in min.	cc. $M/400$ HOCl absorbed per 200 cc. $M/20000$ resorcinol	$k = \frac{2.303}{t(a-b)} \log_{10} \frac{b(a-x)}{a(b-x)}$
None	..	0.1	2.3	9070
None	..	0.25	5.3	10600
HCl	10^{-4}	1.0	2.2	860
HCl	10^{-3}	0.25	6.6	15900
HNO_3	10^{-4}	1.0	2.4	950
HNO_3	10^{-3}	2.0	2.2	430

NOTE:— *Reactants used: HOCl, M/4000; resorcinol, M/20000.*

The same general results were found in studying the effect of hydrochloric and nitric acids on the rate of reaction between hypochlorous acid and phloroglucinol. The results are tabulated in Table XII. In the case of phloroglucinol the uncatalysed rate of reaction with hypochlorous acid is greater than the rate of formation of chlorine at a concentration of hydrochloric acid of 10^{-3}, and in this case hydrochloric acid of this concentration was found to retard the reaction. However, at a concentration of hydrochloric acid of 10^{-2}, where the rate of formation of chlorine becomes of a very high order, a marked catalytic effect is noticed. When no hydrochloric acid is added to the reaction mixture, phloroglucinol absorbs four moles of hypochlorous acid with formation of one mole of hydrochloric acid. In the presence of hydrochloric acid at a concentration of 10^{-2}, one mole of phloroglucinol will absorb seven moles of hypochlorous acid. The end-point of the reaction is fairly definite in both cases.

TABLE XII

ACTION OF HYPOCHLOROUS ACID ON PHLOROGLUCINOL AT 0° C.

Catalyst	Concentration of catalyst	Time in min.	cc. $M/400$ HOCl absorbed per 200 cc. $M/20000$ phloroglucinol	$k = \frac{2.303}{t(a-b)} \log_{10} \frac{b(a-x)}{a(b-x)}$
None	—	0.1	15.1	294000
HNO_3	10^{-3}	0.1	14.2	189000
HCl	10^{-3}	0.1	14.2	189000
HNO_3	10^{-2}	0.1	9.0	45800
HCl	10^{-2}	0.1	19.4	—

NOTE:— *Reactants used: HOCl, M/4000; phloroglucinol, M/20000.*

In the case of phenols, therefore, while hypochlorous acid reacts very rapidly with some phenols, chlorine appears to react more rapidly in all cases so far observed, and the rapid chlorination of phenols by chlorine water is probably due to the presence of nascent chlorine. At least two and possibly more parallel reaction-mechanisms are involved, however, and the whole question of the aqueous halogenation of phenols deserves further study.

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STUDIES ON THE NATURE OF RUST RESISTANCE IN WHEAT
V. PHYSIOLOGY OF THE HOST[1]

By W. F. Hanna[2]

Abstract

Determinations have been made of catalase, diastase and oxidase activity; rate of respiration; and content of chlorophyll, xanthophyll and carotin in the leaves of eight wheat varieties. With respect to their reactions to stem rust, these wheats vary from almost complete susceptibility in certain varieties to a high degree of resistance in others. Catalase activity increased as the plants approached maturity, whereas, diastatic activity decreased with increasing age. No significant differences were found in the oxidase activity or respiratory rate of the varieties. Little Club, and the varieties of the *vulgare* group proved to be relatively rich in chlorophyll and the carotinoids. It is suggested that photosynthetic processes may take place more rapidly in the cells of the varieties having a high content of these pigments, and thus furnish conditions suitable for the growth of the rust mycelium.

Introduction

In the first paper of this series (7) there was presented a general review of the literature on disease resistance in plants. A plan of investigation was also outlined which promised to yield further information concerning the varying degrees of resistance exhibited by varieties of wheat to the black stem rust fungus, *Puccinia graminis tritici*. One part of the proposed investigation dealt with the physiology of the host. It is the purpose of the present paper to present the results thus far obtained on this phase of the work.

Much of the information which has been obtained regarding the physiology of parasitism is based upon the study of saprophytic fungi. Up to the present, the rusts have not been cultured apart from their particular host plants, and little is known of the nutritive requirements of the rust mycelium. However, a certain amount of information on this subject may be deduced indirectly from experiments which have been made on the reaction of the rusts to artificially induced changes in the metabolism of their host plants. Outstanding among these investigations is the work of Ward (11) on the brome rust, *P. dispersa;* and that of Mains (6) on *P. sorghi* and *P. triticina*. Their experiments show that the rusts are dependent for their nutrition on the products of photosynthesis supplied by the host plant, and that when the host metabolism is interfered with by a reduction in the supply of light, carbon dioxide, or minerals, the growth of the rust is correspondingly retarded and sporulation is greatly reduced. A further confirmation of their results is to be found in the observation that, during the winter months when there is a low light intensity, difficulty is experienced in securing good rust infection. The available evidence, therefore, seems to strengthen the hypothesis that the extent

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to which a given form of *P. graminis tritici* will develop on a congenial wheat variety is dependent, primarily, upon the vigor of the host plant.

The importance of host vigor as a factor in determining the varietal resistance of wheats to groups of forms of *P. graminis tritici* has not been investigated. The bread wheats, in general, have proved to be susceptible to stem rust; whereas, the macaroni and emmer varieties are more resistant. It would seem possible that the variations in rust reaction exhibited by groups of wheats, and by individual wheat varieties within a single group, might be attributed, in part at least, to differences in the rate at which their physiological processes are carried on. These differences, in turn, might be expressed in terms of enzyme activity; content of the important pigments such as chlorophyll, xanthophyll and carotin; rate of respiration; and photosynthetic activity.

To test the value of this hypothesis a series of determinations were made of catalase, diastase, and oxidase activity and rate of respiration of fresh leaves collected from eight wheat varieties. Dried leaves of the same varieties were also compared for content of chlorophyll, xanthophyll and carotin. The wheats selected were: Little Club, Marquis, Kanred, Kota, Mindum, Kubanka, Common emmer, and Khapli. In their reactions to stem rust these varieties range from almost complete susceptibility in Little Club to a high degree of resistance in Khapli. In a group of wheats such as this, including as it does, representatives of different species, there might be expected to occur between varieties distinct physiological differences other than those connected with rust reaction. This possibility must be considered in interpreting the experimental results.

Experimental

Catalase

The first series of determinations of catalase activity were made with extracts prepared from plants seeded in the greenhouse January 6, 1926. During the summer of 1926, two series of similar determinations were carried out with plants grown in plots in the field. The first group of plots was seeded May 4, and determinations with material collected from them were made at weekly intervals from June 3 until July 19. At the time of the last collection, the flowering stage had been reached by even the latest varieties. The second group of plots was seeded August 12, and determinations with material collected from them were made August 24, August 31, and September 7.

Preparation of Extract

Freshly collected wheat leaves were cut into pieces about one inch in length, and the pieces were well mixed together. Duplicate 3-gm. portions of each variety were weighed out and placed in a mortar with about 1.5 gm. of clean quartz sand and 1.5 gm. of finely powdered calcium carbonate. About 5 cc. of distilled water was then added from a volumetric flask which previously had been filled up to the mark, and the mixture in the mortar was ground to a thin paste. The contents of the mortar was emptied into an Erlenmeyer flask, and a second 3-gm. portion was ground in the same way and added to

the first one. The mortar was then washed with a little water from the volumetric flask and the washings and the remainder of the water was poured into the Erlenmeyer flask. The latter was shaken, and the contents was allowed to stand for an hour before the catalase determination was made.

Determination

The apparatus employed was similar to that described by Heinicke (2, 3), and consisted of a two-arm reaction tube connected by pressure tubing to the upper end of a burette. The lower end of the burette was connected by rubber tubing to a levelling bulb. The tubing and the bulb contained water, and by raising and lowering the bulb the level of the water in the burette could be brought to the zero mark.

The wheat-leaf extract was shaken, and 4 cc. was introduced with a pipette into one arm of the reaction tube. Into the other arm there was pipetted 5 cc. of hydrogen peroxide (Oakland Dioxygen). The reaction tube was then connected with the rubber tubing leading to the burette, and placed in a water bath kept at 25° C. When the tube and its contents had come to approximate temperature equilibrium with the bath (about 2 min. was sufficient), the pinch-cock at the top of the burette was pressed to permit the water in the burette to rise to the zero mark. The reaction tube was then rocked from side to side, causing its contents to flow from one arm to the other once every second. The time in seconds required to liberate 10 cc. of oxygen was recorded with a stop watch. Duplicate determinations were made with each extract.

Results

Four series of determinations were made on leaves collected at different dates from the plants grown in the greenhouse. The mean of each pair of determinations and the mean of all the determinations for each variety are summarized in Table I. Similarly, the results of the determinations made on leaves collected from the plots seeded in the field May 4 and August 12, 1926, are presented in Tables II and III. The ages of the plants, as given in the tables, were computed from the day of seeding to the day when the leaves were collected.

TABLE I

CATALASE ACTIVITY OF WHEAT-LEAF EXTRACTS PREPARED FROM PLANTS SEEDED IN THE GREENHOUSE JAN. 6, 1926

Variety	Time in seconds required to liberate 10 cc. of oxygen at 25° C.					
	Feb. 2-3	Feb. 4-5	Feb. 8-9	Feb. 10-11	Feb. 15-16	Mean
Little Club	93	65	77	75	64	75
Marquis	76	87	59	59	55	67
Kanred	89	79	63	61	61	71
Kota	96	89	77	59	76	79
Mindum	104	89	81	73	76	85
Kubanka	132	108	87	103	95	105
Emmer	104	114	103	100	89	102
Khapli	132	182	142	110	135	140

There is a tendency for the extracts prepared from wheats seeded in the greenhouse, and those seeded in the field on May 4, to increase in activity as the plants become older. The increase is most evident in the plants seeded in the field. Kanred, a winter wheat, which remained in the seedling condition throughout the summer, changed much less than the others, but nevertheless gave evidence of a slight increase in activity towards the end of the season.

TABLE II

CATALASE ACTIVITY OF WHEAT-LEAF EXTRACTS PREPARED FROM PLANTS SEEDED IN THE FIELD MAY 4, 1926

Variety	Time in seconds required to liberate 10 cc. of oxygen at 25° C.									
	Plants 4 wks. old	Plants 5 wks. old	Plants 6 wks. old	Mean 4–6 wks.	Plants 7 wks. old	Plants 8 wks. old	Plants 9 wks. old	Plants 10 wks. old	Plants 11 wks. old	Mean 7–11 wks.
Little Club	47	50	68	55	94	39	29	23	26	42
Marquis	45	45	62	51	59	25	18	16	15	27
Kanred	43	45	47	45	36	48	30	31	33	36
Kota	61	52	58	57	42	39	19	21	19	28
Mindum	45	46	75	55	46	33	20	18	19	27
Kubanka	70	57	83	70	52	44	22	22	22	32
Emmer	63	65	59	62	45	37	26	18	21	29
Khapli	74	111	82	89	59	47	25	28	33	38

TABLE III

CATALASE ACTIVITY OF WHEAT-LEAF EXTRACTS PREPARED FROM PLANTS SEEDED IN THE FIELD AUG. 12, 1926

Variety	Time in seconds required to liberate 10 cc. of oxygen at 25° C.		
	Plants 2 weeks old	Plants 3 weeks old	Plants 4 weeks old
Little Club	43	59	166
Marquis	51	41	95
Kanred	55	55	82
Kota	53	58	87
Mindum	46	42	91
Kubanka	54	55	104
Emmer	57	71	100
Khapli	115	61	99

The relative positions of the varieties with respect to catalase activity fluctuate somewhat from one determination to another. When the plants were in the seedling condition, Khapli, emmer and Kubanka usually produced the least active preparations. Towards the end of the season, however, there was a decided increase in the activity of these varieties. This seasonal increase in activity is probably determined by the rate at which the varieties approach maturity.

The plants seeded on August 12 gave less active preparations as they became older. This decrease in activity may be attributed to the lower temperatures prevailing at the beginning of September, which retarded plant growth.

Diastase

Portions of the extracts prepared for catalase determinations were used also for the experiments on diastatic activity. The first determinations were made on June 21 with extracts from leaves collected from the plots seeded on May 4. Thereafter, weekly determinations were carried out until July 19. On August 25, August 31 and September 7, a second series of determinations were made on plants seeded August 12.

Determination

The freshly prepared extract was allowed to stand for half an hour, and 10 cc. of it was pipetted into an Erlenmeyer flask containing 50 cc. of a freshly prepared 2% starch solution. The flask was placed in a water bath kept at 50° C. until the contents had reached 35° C. after which it was removed to an incubator and maintained at a temperature of 35° C. for two hours. On removing the flask from the incubator 5 cc. of normal sulphuric acid was added to the mixture to check enzyme activity. The flask was kept in a refrigerator maintained at about 0° C. until the sugar determination was made.

Before carrying out the sugar determinations, the acid in the mixtures was neutralized by adding 55 cc. of *N*/10 sodium hydroxide to each flask. Sugar was determined by the cuprous titration according to the method of Shaffer and Hartmann (10). The following quantities of reagents were used: 10 cc. digested solution, 5 cc. Fehling's A, 5 cc. Fehling's B and 30 cc. of distilled water to make the volume up to 50 cc. for boiling; 5 cc. potassium iodide-iodate solution, 4 cc. 5 *N* sulphuric acid and 5 cc. saturated potassium oxalate. The smaller quantities of reagents gave satisfactory results and reduced considerably the cost of the work. Duplicate sugar determinations were made for each preparation.

To make allowance for reducing substances originally present in the leaf extract and starch solution, or any which might have been produced during the time the flasks were stored in the refrigerator, a control determination was made in the following manner: 10 cc. of each wheat-leaf extract was pipetted into a test tube and placed in the refrigerator. An equal number of Erlenmeyer flasks, each containing 50 cc. of the starch solution, was placed in the incubator at 35° C. for two hours. When the flasks were withdrawn, there was added to each one 5 cc. of the normal sulphuric acid solution, and a 10-cc. portion of extract from one of the test tubes. The flasks were then stored in the refrigerator and, when the sugar determinations were carried out, corrections were made for the reducing substances present in these flasks.

Results

The determinations of diastatic activity are summarized in Tables IV and V. For the purpose of comparing the different varieties, the results have been expressed in terms of the reducing substances produced by one gram of green leaf when added in the form of extract to 50 cc. of 2% starch solution.

Diastase activity, unlike catalase activity, tends to decrease as the plants become older. Kanred, however, is again exceptional in its behavior. The

TABLE IV

DIASTASE ACTIVITY OF WHEAT-LEAF EXTRACTS PREPARED FROM PLANTS SEEDED IN THE FIELD MAY 4, 1926

Variety	Reducing action of 1 gm. of green leaves expressed as mg. of Cu					
	Plants 7 wks. old	Plants 8 wks. old	Plants 9 wks. old	Plants 10 wks.old	Plants 11 wks.old	Mean
Little Club	252	197	143	151	142	177
Marquis	150	146	79	71	54	100
Kanred	340	287	382	416	381	361
Kota	276	223	127	175	138	188
Mindum	166	149	76	84	84	112
Kubanka	194	162	155	187	158	171
Emmer	108	66	34	71	48	65
Khapli	312	242	242	123	103	204

TABLE V

DIASTASE ACTIVITY OF WHEAT-LEAF EXTRACTS PREPARED FROM PLANTS SEEDED IN THE FIELD AUG. 12, 1926

Variety	Reducing action of 1 gm. of green leaves expressed as mg. of Cu			
	Plants 2 weeks old	Plants 3 weeks old	Plants 4 weeks old	Mean
Little Club	591	403	317	437
Marquis	446	252	184	294
Kanred	685	569	414	556
Kota	694	459	372	508
Mindum	592	343	171	369
Kubanka	646	394	248	429
Emmer	420	229	145	265
Khapli	601	358	310	423

preparations made from plants of this variety seeded on May 4 give no indication of diminishing diastatic activity with increasing age, and those from plants seeded August 12 decrease much less rapidly than those from any of the other varieties. Khapli, which was found to have low catalase activity, appears to be high in diastase.

OXIDASE

Some of the extracts prepared for the catalase and diastase experiments were used also to determine the relative oxidase activities of the eight wheat varieties. The determinations were limited to extracts prepared from the plants sown in the field May 4.

Determination

Oxidase was determined according to a method described by Bunzell (1). After the freshly prepared extract had stood for half an hour, 1 cc. of the supernatant liquid was withdrawn and introduced into one arm of a Bunzell tube. Into the other arm there was pipetted 5 cc. of a 1% solution of pyrogallol. A manometer was then fitted into the neck of the tube and adjusted

to make the hole in the stopper come opposite the hole in the tube. The apparatus was attached to a shaker which had been set up in an incubator, and was allowed to stand for half an hour at 25° C., the temperature at which the determinations were to be carried out. The connection between the tube and the manometer was then closed, and the shaker set in motion by means of a small electric motor. The mixture was shaken for 1½ hr., after which the level of the mercury was read. Determinations were made in quadruplicate, and a blank made up with 5 cc. of 1% pyrogallol solution and 1 cc. of water was included with each set of determinations.

The volume of oxygen absorbed by the mixtures was calculated from the volume of the tube in which the reaction was carried out, the depression of the column of mercury and the barometric pressure at the time of the determination.

Results

Oxidase activity has been expressed as the number of cubic centimetres of oxygen absorbed by a mixture of 1 cc. of extract and 5 cc. of 1% solution of pyrogallol, when shaken for 1½ hr. at 25° C. The results are summarized in Table VI. As no correction was made for the carbon dioxide liberated during the reaction, the values do not represent the absolute volumes of oxygen absorbed.

TABLE VI

OXIDASE ACTIVITY OF WHEAT-LEAF EXTRACTS PREPARED FROM PLANTS SEEDED IN THE FIELD MAY 4, 1926

Variety	Cubic centimetres of oxygen absorbed in 1½ hr. at 25° C. by 1 cc. of extract and 5 cc. of 1% pyrogallol solution				
	Plants 4 weeks old	Plants 5 weeks old	Plants 6 weeks old	Plants 7 weeks old	Mean of all determinations
Little Club	0.19	0.18	0.23	0.33	0.23
Marquis	0.21	0.24	0.20	0.25	0.23
Kanred	0.21	0.22	0.29	0.19	0.23
Kota	0.20	0.40	0.21	0.28	0.27
Mindum	0.20	0.21	0.25	0.24	0.23
Kubanka	0.20	0.26	0.22	0.23	0.23
Emmer	0.22	0.32	0.36	0.26	0.29
Khapli	0.23	0.18	0.25	0.27	0.23

All of the preparations proved to be low in oxidase activity and the differences between varieties were in general small. The means for all the varieties are alike, with the exception of Kota and emmer, which show a slightly higher absorption of oxygen. When the errors due to small temperature changes during the reading of the manometer are considered, the differences between varieties would appear to be without significance. It may be concluded, therefore, on the basis of these experiments, that there are no appreciable differences in the oxidase activities of the eight wheat varieties.

CHLOROPHYLL

The material employed in the determination of chlorophyll and carotinoids was collected from plots seeded in the field May 20, 1927. Leaves from these

plots were cut on June 17, and dried at room temperature in the laboratory until they had come to moisture equilibrium with the air. After a further drying of about eight hours in an electric oven kept below 40° C., the leaves were ground in a small mill and the resulting powder was passed through a 60-mesh sieve. This powder was stored in screw top bottles and kept until required for the determinations.

Extraction

Duplicate 1-gm. portions of the leaf powder were placed in 150-cc. Erlenmeyer flasks, and to each flask was added 30 cc. of 85% acetone. The flasks were shaken and left to stand about 18 hr. The extraction was carried out by pouring the contents of each flask on a filter paper fitted in a Büchner funnel. The filtrate was collected in a filter flask connected with a suction pump. When the original 30 cc. of acetone had drained through the filter, the powder was sucked dry. It was then washed with 50 cc. of 85% acetone, added in 10-cc. portions. When all the acetone had drained through, the powder was washed with 50 cc. of ethyl ether, added in 10-cc. portions, and sucked dry. The extract was made up to 200 cc. in a volumetric flask with 85% acetone.

Saponification

A 10-cc. portion of the acetone-ether extract was pipetted into a separatory funnel containing 10 cc. of ether. About 10 cc. of distilled water was then run into the funnel from a wash bottle and, when the mixture of acetone and water had separated out, it was run off leaving the pigments in the upper ether layer. Two more additions of water were made in order to remove all the acetone. The ether layer containing the pigments was then run into a test tube, and traces of the pigments left in the funnel were washed down with about 10 cc. of ethyl alcohol.

The chlorophylls were saponified by adding 1 cc. of 30% methyl alcoholic-potash to the test tube and allowing the mixture to stand about half an hour. The chlorophyllides were separated from the carotinoids by pouring the contents of the test tube into a separatory funnel containing 10 cc. of ether. Distilled water was poured down the side of the funnel until the yellow and green layers separated out. The two fractions were then run out into separate beakers, and a few drops of ether was passed through the funnel and collected in the beaker containing the carotinoids. The chlorophyllides were again added to 10 cc. of ether in the separatory funnel to remove the last trace of carotinoids. The ether separating out on the top was usually colorless. The chlorophyllides were run into a 100-cc. volumetric flask, and the carotinoids which had been drawn off after the first separation were added to the ether remaining in the funnel. Distilled water was added to the ether solution to wash out any chlorophyllides. This water was then run off into the 100-cc. volumetric flask containing the chlorophyllides and the volume was made up to 100 cc. with distilled water.

Determination

Using the solution prepared from Khapli leaf powder as a standard, the

relative concentrations of chlorophyllides in the solutions prepared from the other varieties were determined colorimetrically in a Leitz-Buerker colorimeter. The chlorophyll content of the Khapli solution was determined later by comparing it colorimetrically with a chlorophyllide solution made from a solution of known chlorophyll concentration. The chlorophyll used in making up this solution was extracted from a quantity of Kanred leaf powder, and was purified according to the method of Willstätter, as given by Jörgensen and Stiles (5). A solution of the purified chlorophyll was prepared in ethyl alcohol so as to contain 0.201 gm. of chlorophyll in 100 cc. of solution. A 5-cc. portion of this solution was saponified with methyl alcoholic-potash, and diluted with distilled water so as to have a concentration equivalent to 0.008375 mg. of chlorophyll per cubic centimetre of solution. A 10-cc. portion of the acetone-ether extract of Khapli leaf powder was then saponified, and the resulting solution of chlorophyllide was made up to 50 cc. and compared in the colorimeter with the solution containing the equivalent of 0.008375 mg. of chlorophyll per cubic centimetre. From this reading it was possible to calculate the chlorophyll concentration in the leaf powder of each of the eight wheats. The results have been expressed in Table VII as milligrams of chlorophyll per 100 gm. of dry and green leaf.

TABLE VII

CHLOROPHYLL AND CAROTINOIDS IN LEAVES OF WHEAT SEEDLINGS

Variety	* Age of plants in weeks	Per cent dry matter in green leaves	**Per cent solids extracted from leaf powder by acetone and ether	**Pigments in 100 gm. leaf powder (milligrams)			Pigments in 100 gm. green leaves (milligrams)		
				Chloro-phyll	Xantho-phyll	Caro-tin	Chloro-phyll	Xantho-phyll	Caro-tin
Little Club	3	14.9	30.3	1230	178	98	183	26.5	14.6
Marquis	3	15.9	28.4	1249	176	119	199	28.0	18.9
Kanred	3	15.9	30.8	1179	151	85	188	24.0	13.5
Kota	3	15.7	27.9	1077	149	85	169	23.4	13.3
Mindum	3	15.3	30.2	934	132	76	143	20.2	11.6
Kubanka	3	14.6	27.7	942	121	53	138	17.7	7.7
Emmer	3	16.7	28.5	1061	129	76	177	21.5	12.7
Khapli	3	15.5	28.8	966	130	67	150	20.2	10.4

**From date of emergence.*
***Oven-dry basis.*

XANTHOPHYLL AND CAROTIN

The leaf powder prepared for the chlorophyll determinations was used also for the colorimetric determination of xanthophyll and carotin.

Extraction

A 1-gm. portion of the leaf powder of each of the eight varieties was extracted on filter paper in a Büchner funnel. The funnel was fitted in a filter flask which in turn was connected with a suction pump. Each extraction was made with 100 cc. of ethyl ether added to the leaf powder in 10-cc. portions. After each addition of ether, suction was applied and the leaf powder was sucked dry. The filtrate was then made up with ether to 100 cc. in a volumetric flask.

Separation of Carotinoids from Chlorophyll

As both xanthophyll and carotin oxidize readily when dissolved in ether, it is necessary to separate the two pigments and to determine their concentrations as soon as possible after the extraction has been made. The separation of the carotinoids from the chlorophyll was effected by pipetting duplicate 5-cc. portions of the ether extract into test tubes immediately after the extraction had been made, and adding to each test tube 1 cc. of methyl alcoholic-potash. When saponification had taken place, the contents of each test tube was transferred to a small separatory funnel to which about 5 cc. of ether had been added. The carotinoids were then freed from the chlorophyllides by repeated washings with distilled water.

Separation of Xanthophyll from Carotin

As both xanthophyll and carotin are soluble in ether it was necessary to evaporate off the ether before the two pigments could be separated with methyl alcohol and petroleum ether. This was effected by fitting in the top of the separatory funnel a rubber stopper in which two holes had been made. A small glass tube was inserted in one of the holes, the lower end of the tube almost touching the surface of the ether; the upper end of the tube was then connected with a suction pump. By applying suction the ether was soon completely removed, its rapid evaporation serving also to maintain the contents of the funnel at a low temperature, thus reducing the loss of pigments through oxidation.

After the evaporation of the ether, the pigments remaining in the separatory funnel were dissolved in 10 cc. of petroleum ether (b.p. 30-50° C.) and 5 cc. of methyl alcohol. The mixture was shaken and, when the two layers had separated out, the lower layer containing the xanthophyll was drawn off. This solution of xanthophyll in methyl alcohol was washed with small portions of petroleum ether until the latter showed no color. The solution of carotin in petroleum ether was similarly washed with methyl alcohol to remove any trace of xanthophyll. The combined methyl alcohol washings containing the xanthophyll were made up to 50 cc. The carotin contained in the petroleum ether was made up to 10 cc.

Determination

Standard solutions of xanthophyll and carotin were made up from the pure pigments isolated from the leaf powder of Kanred. The methods employed in the extraction and purification of the pigments were those given by Willstätter with certain modifications introduced by Schertz (8, 9). The xanthophyll was recrystallized five times from chloroform by the addition of petroleum ether (b.p. 40-60° C.). From the crystals so obtained a standard solution was made up with methyl alcohol to contain 0.001 mg. of xanthophyll per cubic centimetre. The carotin was recrystallized twice from chloroform by the addition of small quantities of ethyl alcohol. A standard solution of the carotin was made up with petroleum ether to contain 0.00283 mg. of the pure pigment per cubic centimetre.

The xanthophyll solutions made up to 50 cc. in methyl alcohol, and the carotin solutions made up to 10 cc. in petroleum ether were compared in the Leitz-Buerker colorimeter with the standard solutions of xanthophyll and carotin. From the readings obtained the concentration of the two pigments in the green and dry leaves were calculated. These have been expressed in Table VII as milligrams per 100 gm. of leaf.

Results

The results of the determinations summarized in Table VII show that Little Club and the three varieties belonging to the *vulgare* group are relatively rich in leaf pigments, particularly the yellow pigments, xanthophyll and carotin. Little Club and Marquis have the highest content of yellow pigments and, when the results are calculated on a dry weight basis, they have also the highest content of chlorophyll. When the pigment content of the green leaves is considered, Kanred is slightly richer in chlorophyll than Little Club.

Respiration

Methods

The leaves used for determining the rates of respiration of the eight wheat varieties were collected from plants grown in small plots in the greenhouse. In these experiments Vernal emmer was substituted for Common emmer. So as to have the plants of all varieties at as nearly as possible the same stage of development at the time of the determinations, the seedings were made between June 23 and July 1, one variety being seeded each day. When the plants of each variety were about two weeks old, leaves were collected, and determinations were made of their rates of respiration. The dates of seeding and the time of each determination are given in Table VIII.

The apparatus set up for the determination of the carbon dioxide given off by the leaves consisted of an electrically controlled incubator maintained at 25° C., in which the chamber containing the leaves was placed; a number of solutions for washing the incoming air; and a Reisset tower for collecting the carbon dioxide given off by the leaves. The suction necessary to draw the air through the system was supplied by a small electrically driven vacuum pump, which was connected with a mercury manometer to ensure a constant circulation of air. Before entering the chamber, the air passed through a gas washing bottle (300 cc.) containing a concentrated solution of potassium hydroxide; a second gas washing bottle containing sticks of potassium hydroxide; and a Reisset tower containing 100 cc. of 0.17 *N* solution of sodium hydroxide. After passing out of the Reisset tower, the air bubbled through 200 cc. of sulphuric acid solution (sp.gr. 1.24); this brought the air to a relative humidity of approximately 70% before it entered the incubator. Within the incubator, the air circulated through a coil of copper tubing and was thus brought to a temperature of 25° C. before it entered the glass bottle of 140 cc. capacity which contained the leaves. The carbon dioxide given off by the leaves was absorbed in a Reisset tower containing 100 cc. of 0.17 *N* solution of sodium hydroxide. During the experiment the leaves were kept in darkness.

About 4.5 gm. of leaves collected from the plants grown in the greenhouse was placed in the respiration chamber, and the air was drawn through the system for 20 min. The motor was then stopped for 5 min. and, during this time, 100 cc. of the 0.17 *N* sodium hydroxide solution was run into the Reisset tower in which the carbon dioxide was to be absorbed. The air was again circulated for one hour and 55 min., making in all a period of two hours during which the carbon dioxide output was measured.

The two-hour period for collecting the carbon dioxide was arrived at by a preliminary experiment. About 4.5 gm. of green leaves was placed in the respiration chamber and the amounts of carbon dioxide given off during the first, second and third hours were determined separately. The titration of standard hydrochloric acid equivalent to the carbon dioxide absorbed during each period was: 1st hour, 2.53 cc.; 2nd hour, 2.61 cc.; 3rd hour, 2.33 cc. A second experiment with another portion of approximately 4.5 gm. of green leaves from the same variety gave the following titrations: 1st hour, 2.68 cc.; 2nd hour, 2.70 cc.; 3rd hour, 2.30 cc. These results indicate that at 25° C. the leaves maintain a fairly constant rate of respiration for the first two hours, but that when kept at this temperature for a longer period the output of carbon dioxide begins to fall off.

TABLE VIII

RATE OF RESPIRATION OF LEAVES OF WHEAT SEEDLINGS, EXPRESSED AS MILLIGRAMS OF CARBON DIOXIDE PER HOUR PER GRAM OF DRY LEAF

Variety	Date seeded	Determination of respiration			
		Date	Time	Rate*	Mean* rate
Little Club	1–7–27	21–7–27	9.27–11.27 a.m.	4.80	
	1–7–27	21–7–27	11.50– 1.50 p.m.	4.33	
	1–7–27	21–7–27	2.12– 4.12 p.m.	4.10	4.41
Marquis	30–6–27	20–7–27	9.22–11.22 a.m.	3.48	
	30–6–27	20–7–27	11.44– 1.44 p.m.	3.94	
	30–6–27	20–7–27	2.06– 4.06 p.m.	4.34	3.92
Kanred	24–6–27	12–7–27	9.47–11.47 a.m.	3.89	
	24–6–27	12–7–27	12.44– 2.44 p.m.	4.03	
	24–6–27	12–7–27	3.25– 5.25 p.m.	4.20	4.04
Kota	27–6–27	15–7–27	9.35–11.35 a.m.	4.56	
	27–6–27	15–7–27	12.37– 2.37 p.m.	4.45	
	27–6–27	15–7–27	3.01– 5.01 p.m.	5.40	4.80
Mindum	25–6–27	13–7–27	9.52–11.52 a.m.	4.43	
	25–6–27	13–7–27	12.17– 2.17 p.m.	4.83	
	25–6–27	14–7–27	2.49– 4.49 p.m.	4.58	4.61
Kubanka	28–6–27	18–7–27	9.45–11.45 a.m.	4.14	
	28–6–27	18–7–27	12.09– 2.09 p.m.	4.31	
	28–6–27	18–7–27	2.33– 4.33 p.m.	4.58	4.34
Vernal emmer	23–6–27	11–7–27	10.04–12.04 p.m.	4.38	
	23–6–27	11–7–27	1.08– 3.08 p.m.	4.36	
	23–6–27	11–7–27	4.06– 6.06 p.m.	4.05	4.26
Khapli	29–6–27	19–7–27	9.15–11.15 a.m.	3.68	
	29–6–27	19–7–27	11.39– 1.39 p.m.	3.55	
	29–6–27	19–7–27	2.03– 4.03 p.m.	3.44	3.56

**Milligrams of carbon dioxide per hour per gram of dry leaf.*

The carbon dioxide given off by the leaves and absorbed by the solution of sodium hydroxide was titrated with a standard hydrochloric acid solution. In making the titration, the excess sodium hydroxide was first neutralized with approximately 0.18 *N* hydrochloric acid, using phenolphthalein as indicator. During this titration, the flask containing the solution was shaken vigorously so as not to decompose any of the bicarbonate. When the neutral point was reached, 14 drops of B.D.H. (British Drug Houses) General indicator was added to the solution, and the titration was continued with 0.0464 *N* hydrochloric acid until the solution reached a pale red. From this titration the amount of carbon dioxide given off by the leaves during the two-hour period was calculated. The leaves used in each experiment were placed in an aluminum dish and dried over night in an electric oven at 80° C.; final weights were made after further drying at 98° C., in vacuo, for 48 hr. Determinations were made in triplicate on each variety. The first determination each day was made from about 9.30 to 11.30 a.m.; the second from about 12 a.m. to 2 p.m.; and the third from about 2.30 to 4.30 p.m. The results have been reported in Table VIII as milligrams of carbon dioxide, per hour, per gram of dry leaf.

Results

In interpreting the results of the respiration experiments several factors should be kept in mind: the relation between cell contents and inert substances such as cell walls and mechanical tissue; the amount of respirable material; and the conditions of light, temperature, and humidity to which the leaves were exposed previous to the determinations. From the results presented in Table VIII it will be seen that the variation in the respiration rate of different collections of leaves from the same variety is frequently greater than the differences in the means for varieties. Khapli presents an exception in that it gave a relatively low respiration rate in all three determinations. Further work, in which all the conditions of the experiment would be carefully controlled, might bring out small but characteristic differences in the respiration rates of these wheats.

Discussion

The results of the foregoing experiments indicate that some of the physiological properties of wheat leaves may be affected quantitatively by the conditions under which the plants are grown. The age of the plants is also an important factor in determining physiological activity. These facts must be considered in evaluating the differences between varieties or groups of varieties.

The catalase activity of the varieties studied increased as the plants approached maturity, whereas, diastatic activity decreased with increasing age. Khapli, emmer, and Kubanka had low catalase activity in the seedling stage of growth, but towards maturity there was little difference between them and the other varieties. Emmer had the lowest diastatic activity of any of the varieties studied, with Marquis coming second; while Little Club, a susceptible variety, and Khapli, which is extremely resistant, had much the same order of diastatic activity.

Little Club, and the varieties belonging to the *vulgare* group proved to be relatively rich in the green and yellow leaf pigments. This relationship is of considerable interest, because it is among such wheats that the varieties most susceptible to stem rust are found. Hursh (4) has already suggested that in rust resistant wheats the ratio of mechanical tissue to chlorophyll-bearing tissue is relatively high. The fact that certain varieties of wheat are found by analysis to be comparatively rich in chlorophyll and carotinoids, may indicate only that they are correspondingly poor in mechanical tissue. It is also possible, however, that in the individual chlorophyll-bearing cells of such varieties there is a high concentration of the important pigments. In tissues such as these photosynthetic processes would take place rapidly, and thus furnish conditions suitable for growth of the rust mycelium. Further research on the carbohydrate metabolism of these wheats might throw new light on the nature of rust resistance.

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A STUDY OF THE MALE GENITALIA OF CANADIAN SPECIES OF PENTATOMIDAE[1]

By Alex. D. Baker[2]

Abstract

A description of the male genitalia of the Pentatomidae is given, followed by a comparative study of these structures as found in Canadian species of this family. The development of our knowledge of the subject is indicated and the more important papers reviewed in the light of the author's findings. Each of the genital structures is discussed and described in turn. The act of copulation and the workings of the genital parts are described. Descriptions of a number of Canadian species of Pentatomidae are given in detail. A section is devoted to an explanation of the general line of development of the genital structures within the family.

I Introduction

This paper is intended primarily as a contribution to the morphology of the male genital structures of the Pentatomidae. Incidentally it may offer some suggestions as to the relationships of the families of the order.

Study was made of a wide range of literature dealing with the genitalia of other groups that appeared to have any features in common with the genitalia of the Pentatomidae. This was done in order that a comprehensive understanding of the homologies of this group could be obtained. Part of this paper is thus devoted to a discussion in which the essential structures of the male organs of generation in insects are briefly reviewed, with particular regard to an understanding of the structures in the Pentatomidae.

Before a comparative study was possible, a general study of the genitalia of this family was essential. As a result the questions of homologies and nomenclature receive considerable attention, but the formation of new terms is avoided as far as possible.

In specific descriptive notes the organs are described as they exist *in situ* and differences in color are not stressed.

In the Pentatomidae, as in all other Hemiptera, the sexes are usually recognized by the structure of the seventh sternum and those segments caudad to it. In the male, the seventh sternum is complete. In the female, the basal part of the seventh sternum has a median division, forming two lateral plates, while the apical region is split into several smaller plates. Secondary sexual characters are not evident.

This study is restricted to such Canadian species as were available. Some difficulty was experienced with the identification, but I am satisfied that the determinations finally arrived at are the correct ones. In doubtful cases I have been to considerable trouble to confirm the identities of the specimens. This identification of species entailed a study of all keys and descriptions of species that were available. No single work included keys to all the species listed in Canada, and many keys naturally included numerous genera and species which do not occur in this country. In consequence, it was finally thought advisable and convenient to compile keys for our Canadian species. These have been published elsewhere.

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The purpose of this work, then, is mainly to attempt to explain that which has not been explained before. The taxonomic value of the genital structures has been recognized now for some time by systematic students of insects. In the group studied we have a wide range of differences which make the majority of the species easily discernible. That specific differences should not be based on the genital characters alone is recognized, but in some groups of insects they are particularly useful and efficient. The Pentatomidae is one of these groups.

II Historical

The available literature dealing with the male genital structures of the Pentatomidae was found to be surprisingly small at the time this work was first written. While general studies have been made by many workers, who found the male clasping organs, etc., of value in the identification of a few species, this information was usually very meagre and scattered.

One publication was found to be of particular value. This was the paper by David Sharp (1890) "On the structure of the terminal segment in some male Hemiptera". Sharp recognized a "terminal chamber" (genital-cup), in which he located the following structures, *viz.*,

1. The aedeagus.
2. The rectal-cauda, which he describes as being free and very mobile.
3. Some accessory pieces or appendages, *viz.*, (a) lateral, one on each side; (b) inferior, a single piece.

The inferior appendage he refers to is apparently merely an elevation of the ventral border of the genital-cup adjacent to the distal extremity of the rectal-cauda (proctiger). The lateral appendages, he states, "are in many species very mobile, though in other cases very little power of movement appears to be present". I have not observed any specimens where these structures were not capable of movement. The theca he defines as "a tubular or cylindrical structure fastened to the inner face of the floor of the chamber, and completely surrounding the aedeagus; it is, in fact, a fence or hedge, open only above." Structures which he termed the "superior lateral processes" he defined as follows: "There frequently exists on each side of the 'diaphragm', and close to the rectal-cauda, a projection of variable form." These superior lateral processes are apparently the genital plates of this paper.

While Sharp's study does not include any of our Canadian species of Pentatomidae, there are several observations made by him regarding structures which are also present in our species here. The "inferior process", which is referred to above, he describes as being articulated in certain species (*Tesseratoma* sp.), and states "that co-ordinated action between the inferior and the lateral appendages perhaps exists." This inferior process is not an appendage, but, I believe, a simple outgrowth or outfolding of the ninth sternum, and if it is capable of any movement it must be very limited indeed.

In *Nezara* sp., a process projecting backwards over the base of the rectal-cauda Sharp describes as being a projection of the anterior chamber projecting backwards, and he homologizes this projection with the folded and corrugated

membrane surrounding or, more properly, forming the base of the rectal-cauda in other species. This homology is not indicated herein.

The "diaphragm" is defined as a part of the genital structures which descends from the dorsal region more or less obliquely, and forms the separating structure between the "terminal chamber, which is open above, or both above and behind," and the "anterior chamber, which is covered in." Further, in the concluding remarks, it is stated, "that it appears more probable that the original termination of the genital segment was at the line of the diaphragm, and that the part posterior to this,—that is, the posterior chamber, in which the external male parts are situated— is a subsequent growth that has taken place *pari passu* with the evolution of the male parts for the purpose of their protection." In my discussion on the evolution of these parts it will be seen that a different view is presented.

Of the rectal-cauda (proctiger) it is said, "I have stated, in speaking of the rectal-cauda, that in many forms it does not extend to the extremity of the body, but terminates in the middle of the genital segment. This certainly is a very curious arrangement, and at present I am not able to state any incontrovertible reason for the abbreviation. Evidently the cauda, *i.e.*, the alimentary canal, ought to extend to the extremity of the body, for it does so in the female Pentatomidae in a conspicuous manner, and it does so in the males in many species. It would hence appear that its abbreviation in some forms must be looked upon as a departure from the natural arrangement of the parts. And it must be considered a very peculiar departure, for it is difficult to understand how in such cases the excrementitious matter is extruded entirely from the body. Mr. Champion tells me that he is under the impression that some bugs have the power of forcibly ejecting the excrementitious matter by a sort of squirting process; but if this is the method employed in the Scutellerinae, it must be accompanied by a process of spreading the wings to get their tips out of the way, while at the same time the genital segment must be greatly exerted." An explanation of this apparently inefficient structure is suggested elsewhere in this paper.

The functions of the "claspers" have been described by Sharp. He states, "I see no reason for considering, with any great probability, that any part of the structures are clasping or holding instruments. I look on them as: (1) for the protection of the sensitive parts from pressure; (2) for the exclusion of parasites; (3) as directing instruments to determine the exact direction of movement of the true intromittent organs; and (4) as probably instruments for altering the pressure on the ejaculatory canal at its point of entrance into the aedeagus; but the superior lateral processes, which are present only in some species and are always fixtures, may be of the nature of supports or holdfasts." The views of the writer on this point are included in those parts of this paper dealing with the possible homology and evolution of these structures.

Of the aedeagus it is stated, "that I cannot with certainty homologize the parts in some of the forms", and "I would suggest that it may ultimately prove to consist of three parts, *viz.*, two lateral lobes similar to one another, and a

single median ligula or style." The movements of the aedeagus were apparently somewhat obscure as it is observed that, "The aedeagus in the Pentatomidae is not capable of being thrust out of the body as it is in the Coleoptera", and again, "if it exercises any movement during copulation—which appears to me very doubtful—it must be that of depression or elevation of the part external to the transverse joint." In some cases, at least, I have shown herein, that the aedeagus is capable of extrusion from the genital segment. Whether the lateral appendages (claspers) have any controlling power over the passage of the seminal fluid I consider to be very doubtful, although I was at first also inclined to this view.

Since this paper was written, and prior to publication, other works have appeared bearing on the problem. These are referred to in the discussion in the fourth section of this paper.

III Technique Adopted

It will be noticed that the genital structures normally visible *in situ* are emphasized. This makes possible the more practical application of the structures described for purposes of easy and yet certain identification. Where it is necessary to dissect and generally mutilate an insect for purposes of identification, the practical value of the genital structures is greatly lessened. In those cases where more or less elaborate methods of technique are required to prepare and mount the structures, the practical value of the parts to the taxonomist becomes still more lessened. All methods may be necessary for purposes of study and determination of specific characters, but all are not of equal practical valŭe. In the specific description an attempt has been made to make use of those structures of the more practical order wherever possible.

Drawings of the genital segment from the central aspect, as well as including the adjacent abdominal segments, show the extent to which the wing tips project caudad. In *Coenus delius* it will be noted that the wing tips are not normally visible from the underside of the insect.

Bethune-Baker (1914) states: "The natural and best position for observation of reproductive structures is the profile view in the Lepidoptera, Trichoptera, Neuroptera, and Diptera. The organs are co-ordinated together as nature made them, as they are used by the insects themselves, so that the relation of part to part is at once apparent to the eyes of the observer; vertical and flat, transparent and opaque are all needed sometimes when it may be possible, but the educative and natural position is the profile." The difficulty of illustrating the genital structures from the best position was pronounced. In the majority of the specimens the copulatoria lies within a concavity or "cup" formed by the ninth segment. A lateral drawing would show little or nothing of the retracted structures. A ventral view would often show only the outline of the "ventral" border of the genital-cup and the ventral surface of the exposed portion of the segment. A caudal view, in many cases, would be the most satisfactory aspect, but in some groups, *e.g.*, *Euschistus* sp. the dorsal aspect is the best. In drawings from the dorsal aspect, however, it was found that the seventh tergum covered a large part of the opening of the genital-cup, and

that only from a dorsal-caudal aspect could the interior structures of the cup be observed at all satisfactorily. It was thought best, in order to compare adequately the various species, to draw them all from the same aspect. The caudal aspect was the one selected and a direct caudal view was drawn of all the species examined. In addition, other aspects were selected where it was thought necessary, particularly those from the ventral aspect, the dorsal aspect, latero-caudal, and to a limited extent, the lateral aspect. As mentioned above, where drawings from the ventral aspect were made, the relation (but not the details) of the wing tips were included.

Inclusion of the abdominal segments immediately adjacent to the genital-cup were made for the purpose of showing the relative position and size of the genital segment.

In the drawing of the genital structures the inclusion of hairs was reduced to a minimum, and entirely excluded whenever it was thought advisable. The position, kind, density, etc., of the hairs was generally included in the descriptive notes, and where there were conspicuous and specific growths they were included in the drawings. To include all vestiture in the drawings would, in many cases, make for obscurity of many of the more important morphological differences which it was tried to emphasize. The type of genital structures found in this family make this point a very important one, and the inclusion of the vestiture, which is so frequently found around the borders of the genital-cup, would have rendered it extremely difficult to depicture satisfactorily the details of the important structures lying within the cup.

For the same reasons as those advanced above, line drawings were used as much as possible, with shading and stippling reduced to a minimum. Occasionally this general rule was somewhat departed from, particularly where prominent depressions, etc., occurred which it was thought necessary to figure and, again, where it was necessary to shade in order to make one part stand out more clearly from another.

In a few of the drawings a slight asymmetry of parts may be noticed. This was necessary where there appeared to be slight differences between individuals of the same species or slight differences within the individual. In addition, the relative position of the claspers might not always be the same from the same aspect. This latter point will be understood better on perusal of that part of this paper dealing with the working of the genital structures.

Oil-like secretions on the cuticle, etc., often rendered the structures obscure and gave false impressions of the outlines of the different parts. This had to be guarded against, as where such secretions were present, elevations, tubercles, etc., appeared to be quite apparent until touched with the needle point. To overcome this the parts were gone over with a camel hair brush saturated with benzine naphtha. This procedure was found to be fairly satisfactory, and also very necessary, as these secretions are very prevalent in insects of this group.

Drawings of the complete genital structures from the dorsal, ventral or caudal aspects were all made to the same scale of magnification. This was thought best, in order to make comparison easier. In the drawings accom-

panying the specific descriptions only two scales of magnification were used. The greater magnification is twice that of the lesser.

For details of the genital structures, particularly those of the penis, the intersegmental apodemes, and the relations and working of the various organs, detailed mounts were necessary. For such mounts the usual treatment with potassium hydroxide, etc., was followed. Instead of boiling in the potassium hydroxide, the structures were placed in an electric oven with this medium until the chitinized parts were freed from any adjacent fleshy material. More delicate results were obtained in this way. It was found necessary to stain and also to bleach some material, in order to see the finer structures more clearly.

In transferring small structures from one liquid to another, the best results were obtained by making use of an ordinary match stick, as the smaller structures usually adhered quite readily to the wood, and yet were easily jolted off when placed in the next liquid. With this method no distortion or injury of the parts was possible.

IV Discussion

While eleven segments may be identified in some generalized adult insects, the usual number of visible segments found in adult insects is ten. The anal opening is located in the caudal region of the last abdominal segment. In some generalized insects this would be the eleventh segment; in more specialized insects, the tenth. The folds of membranous tissue surrounding the anus in the insects with the lesser number of segments may, quite possibly, represent all that remains of the eleventh segment.

Reduction in the number of segments in the abdomen may take place through the fusion of certain segments, more commonly the anterior ones, so that two or more segments appear as one, owing to the disappearance of the separating sutures or to an invagination or telescoping of some of the caudal segments. The result is that the number of segments that are visible externally is correspondingly less. As a general rule, the anterior segments of the abdomen are smaller than the others, and the sternum of the first segment is frequently wanting.

From the testes, or manufacturing centres of the male germ cells, come the seminal ducts or vasa deferentia, which are the conveying channels for the products of the testes. The vasa deferentia are normally slender delicate tubes, until they dilate to form an enlarged chamber for the temporary storing of the seminal fluid, to which the name of vesicula seminalis is given. These seminal vesicles may be paired, single or entirely wanting. From the vesicula seminalis, if present, arises a further conducting tube, the ductus ejaculatorius, which is fitted for the passage of the male elements to the exterior of the insect's body, and is of ectodermal origin. In some primitive insects this structure is paired, and each tube opens separately to the exterior. In most insects the ductus ejaculatorius terminates in the flexible, more or less membranous, intromittent organ of the male known as the penis.

Closely associated with the penis proper may be found other closely in-

vesting structures to which a host of terms have been ascribed by workers in the various groups of insects, *e.g.*, penis valves, theca, telaedeagus, penisfilum, etc. To avoid confusion, the general term of aedeagus will be used in this paper to designate the true penis and all those structures, such as indicated above, which are closely associated with it.

In addition to the aedeagus, there are usually additional structures, such as prehensory organs to hold the female during copulation, and the various modifications of the posterior segments of the abdomen to accommodate the genital structures. All of these structures, however, being to aid the passage of the reproductive elements and to protect the more delicate structures more immediately concerned therewith, are necessarily genital structures in the broader sense.

It would appear, then, that there are two quite distinct sets of external genital organs, the aedeagus with its various parts, and the external clasping organs with the various modifications of the adjacent abdominal sclerites. The designation of "copulatoria" has been suggested by Dr. Crampton and others for this entire copulatory apparatus in insects.

The study of the genital structures in primitive insects gives us an important index to the more complicated organs found in the more specialized groups. The condition of the male genitalia in the primitive Mayfly, *Blasturus cupidus*, is concisely described by Crampton (1920) as follows:—

"The sternite of the ninth abdominal segment in this insect bears a pair of somewhat closely united, platelike sclerites, called the styligers or 'coxites'. Dr. Walker correctly compares these styligers, or 'coxites' with the basal segments of abdominal limbs (protopodites) retained in such lower insects as *Machilis*, in which the styligers or 'coxites' bear styli which are possibly homologous with the exopodites (or epipodites) of crustacean limbs. Similarly, in *Blasturus*, the styligers or 'coxites' bear styli; but in the latter insect there are traces of two segments in the styli (and in some mayflies there are three or more segments in the styli), while the styli of most apterygotan insects are composed of but one segment. The segmented styli of ephemerids are called gonopods, gonostyli, or arthrostyli."

The origin of the parts of the copulatoria, apart from the aedeagus, is in a great number of groups extremely uncertain; it has been, and is, the ground for considerable argument between men who have given their careful attention to this subject. Whether these objects are modifications of parts of primitive biramous abdominal appendages or secondary specializations of the abdominal segments adjacent to the genital opening, is a subject rather beyond the limits of the present paper. Further embryological work, and the gradual accumulation of data on the various groups, should, in time, furnish the answer. It is probable that both processes have been at work in the formation of these structures. However, it is usually possible to determine the abdominal segment from which certain structures originate. For the clasping organs, found originating from the ninth abdominal segment in the male, I have used the term "clasper". These are probably the styli. The cerci of insects, when present, always arise from the primitive eleventh abdominal segment.

Crampton, (1920) continues: "The tenth tergite overlaps the paraprocts, which are situated on either side of the anal opening and bear the cerci. The paraprocts are latero-ventral structures of the eleventh segment, and are usually interpreted as representing the divided sternite of this segment, although it is possible that they represent the protopodite of the uropod whose endopodite forms the cercus. The eleventh tergite is usually atrophied in the higher forms, while the paraprocts usually unite with the tenth tergite to form a structure through which the anus opens (*i.e.*, the proctiger of higher insects)."

The position of the anus, opening as it does through the very last of the primitive abdominal segments, is usually of considerable value in any attempt to homologize the adjacent structures, although, in many groups, its containing segment has migrated to a greater or less extent from its primitive position.

Dr. Crampton (1920) homologizes the terminal abdominal structures in a Psyllid Homopteran as follows:—"The tergal sclerites labelled 'ep' bears the anus at its tip (*i.e.*, it is a proctiger). The proctiger 'ep' probably represents the tenth tergite united with the ninth, although the embryology of these insects would have to be studied in order to definitely determine what segments enter into the composition of the structures in question. The structure labelled 'g' is here interpreted as representing the united basal segments of the gonopods, though it may also include the ventral plate of other insects as well. The forceps 'h' apparently represents the distal segments of the gonopods. All that remains of the phallus or aedeagus, is the slender bowed structure 'pv', which bears a terminal articulated appendage or 'telaedeagus' fitting into the groove on the lower (posterior) surface of the proctiger 'ep'."

While these structures appear to have several points somewhat in common with those found in the group under consideration here, if we are to homologize the two types the identification of the parts as expressed above would seem to be somewhat at variance with those expressed in this paper. The writer has not made a study of these parts in the Psyllids and no criticism is intended, but from the study of the homologous (?) structures in the Pentatomidae it might appear improbable that the ninth tergum enters into the composition of the structure labelled the proctiger, and that it possibly represents all that remains of the tenth and rudimentary eleventh abdominal segments. The structures labelled "g", I would suggest, have possibly been largely made up from the ninth abdominal segment. From the drawing it would appear as if the sternum was all that remained of this segment.

The structures referred to as the telaedeagus in the figure referred to above, and as the penisfilum in the drawing of the Mecopteran insect *Bittacus pilicornis*, would appear to have possible interesting homologies in the penisfilum found in several species of Pentatomidae (of which more will be said in the discussion of the aedeagus in this paper).

The part termed the proctiger in the two drawings last mentioned would appear to be homologous with that termed the "rectal-cauda" by Sharp (1890). Exact homology with the "uncus", "tegumen", "pygidium", "epiproct", etc.,

in other groups is difficult, but they are all possibly largely derived from the primitive tenth segment of the abdomen. This question is further complicated by the use that is made of these terms by others who have studied this problem, particularly from other angles.

The above description and discussion is intended as an outline of the essential structures of the male organs of generation, with special regard to those found in the study of the male genitalia of the Pentatomidae. More will be said regarding the homology of the various parts in the writer's descriptive notes on this group, and again in that part that deals with a suggested line of development of these parts.

The present paper was first written some years ago. At that time the writer did not have the assistance of several excellent papers that have since appeared, which relate somewhat closely to the subject in hand. I refer particularly to the papers by Crampton (1922), Hem Singh-Pruthi (1925), and Muir (1926, etc.). It may have been of some advantage to have arrived at my conclusions independently and without the bias that might have been built up by these convincing writers. Their contributions to the subject are marked and they have been of considerable interest to me in comparing results. We agree on many points; but there are also many points on which we do not agree. I feel that this paper would not be complete if I did not review our findings. It is my aim to make my criticisms constructive.

Readers of Crampton's very interesting paper (1922) will find that we are in agreement on many points. My findings do not agree with his theory regarding the origin of the "hypandrium". The structures labelled "hypandrial valves" are probably homologous with the genital plates described herein. As the evolution of the genital-cup has progressed, these structures have come to lie within that organ. The genital plates are apparently included in one of Crampton's drawings, where a hypandrium has been formed (Fig. 19), but have not been named. These structures are still more conspicuous in *Apateticus bracteatus*. It is not my belief that the hypandrium has been formed from the parts termed the hypandrial valves in insects where a hypandrium is absent. The structures termed the aedeagus and penis are as herein. The telaedeagus I have termed the penisfilum.

The paper by Hem Singh-Pruthi (1925) requires considerable comment. It is a large and very important work, representing a considerable advance in our knowledge of the subject, but we differ on quite a few points. Unfortunately, many of my remarks must necessarily be confined to our differences.

In describing the genital-cup, Singh-Pruthi speaks of the "segmental membrane which, especially in Heteroptera, forms a pouch in which the organs (copulatoria) lie." I do not think that this "genital chamber" (herein termed the genital-cup) is formed from the segmental membrane. My views regarding the formation of this structure are given in that section of this paper dealing with a suggested line of development. Further, the inferior process thus does not arise from the segmental membrane.

The aedeagus is described as "a backward continuation of the body wall between the IXth and Xth sternites—that is, of the segmental membrane" (p. 132). The aedeagus, through its basal plate and connectives, is definitely linked with the primitive posterior margin of the ninth sternum, as are the claspers, but the latter frequently have their own connection, which is quite remote on either side of the connection of the aedeagus.

The connection between the aedeagus and the claspers is not as close as might be assumed (p. 133, par. 3). It is true that there is some connection, but it is largely muscular, with slight evidences of sclerotizations. (See descriptions of aedeagus, etc. in the present paper.)

Sharp's older term of "theca" is retained for what Singh-Pruthi terms the "phallosoma", except that it is herein designated the "penial theca" in order to avoid any confusion with several other structures to which the former name has been applied. The "endosoma" are herein represented by the penis lobes, the lateral lobes of which are the same as the "conjunctiva", of which the median lobe, with the penisfilum, when present, is the same as the part termed the "vesica" by Singh-Pruthi. I have not noticed the fusion of the penis lobes with the seminal reservoir (p. 133, par. 4). Singh-Pruthi's description of the "phallosoma mouth" fits the condition found at the distal end of the penial theca (p. 134, par 2). On p. 134 he states "the three regions, phallosoma, conjunctiva, and vesica, are often imperceptibly continuous into one another." And again, "sometimes the endosoma is membranous throughout and undifferentiated into conjunctiva and vesica." I agree with these conditions, and think it preferable to refer to these structures as the lobes of the penis. Crampton also terms all of this structure the penis. The structures herein termed the titillators correspond to what Singh-Pruthi calls the "phallosoma appendages."

The basal plate, as herein described, when bleached and stained, has the superficial appearance of a single structure bounded by areas which correspond to the "basal plates" and "basal plates bridge" of Singh-Pruthi. The ejaculatory duct passes through the basal plate, being bounded by the above-mentioned sclerotizations as it enters the median region, termed the "basal plates prolongation" by Singh-Pruthi. The attachments of the complete basal plate are described herein. Singh-Pruthi did not investigate the attachments of this basal structure to any detailed extent. I feel this is an important feature.

In regard to Singh-Pruthi's discussion of the works of Muir and Kershaw (p. 141), the genital plates, as herein described, arise from the ninth sternum (?). I might suggest that they may represent the "subgenital plates" of Homoptera.

Unfortunately I have not had the opportunity to examine any of the species of Pentatomidae Singh-Pruthi worked with, so I can only make general statements in reference to that section of his paper dealing with the "Special Morphology." The lateral basal plate sclerotizations of the Pentatomidae could hardly be described as being "rod-like" (p. 149, bottom).

I agree that the general plan of the genitalia in the Coreidae (p. 154), is the same as that of the Pentatomidae. The parts are fairly easy to recognize and quite alike in many respects.

On p. 186 Singh-Pruthi states, "The parameres are connected to the basal plates by means of ligaments, but are not in actual contact with the same." He is correct in saying that there is no actual contact, and the connections are by no means strongly or even clearly sclerotized.

On p. 186 it is said that the "basal plates are thin, flat, simply touching, not fused with each other in the mid-ventral line," but on p. 149 (bottom) they are described as being "rod-like." I feel that there is some confusion here between sclerotized sections of the plate and the ventral connectives.

The "ejaculatory duct within the vesica" I do not think can always be described as "thicker than in the phallosoma region." In the structure herein termed the hinge it is often quite faint, but is frequently strongly sclerotized where it passes through the penial theca (phallosoma). The inferior process is not always present as stated. (See my specific descriptions.)

On p. 238 Singh-Pruthi states, "The Xth segment being extremely small, the intersegmental membrane between this and the IXth—the segmental membrane—is very large." And "The genital organ proper (the aedeagus) originates from the ventral region of this area near the posterior margin of the IXth sternite." If the part he terms the "genital chamber" is lined with the intersegmental membrane (see also p. 132, par. 4), the ventral border of the IXth sternite must be considerably removed from the aedeagus. I do not think it is. Further, the arguments advanced, "if the segmental membrane is the IXth sternite", cannot be directed here, as this region is herein described not as the ninth sternum but an invaginated portion of it. Not having made a detailed study of the development of Homoptera, I do not feel entitled to enter the "morphological paradox" argument, unless invited.

Singh-Pruthi concludes that the subgenital plates are "nothing but the prolongations of the IXth sternite" (p. 240). The genital plates, of this paper, fit this section of his description.

In referring to the work of Crampton on p. 243, it is stated by Singh-Pruthi that "the styles in Orthoptera are at the posterior margin of the IXth sternite, while the parameres in Rhynchota are at an entirely different place, connected at the base and lying on the sides of the aedeagus." As will be seen from my studies, these organs are located at, and strongly connected to, the posterior margin of the ninth sternum. There is some misunderstanding here also, as Singh-Pruthi states on p. 238 that the aedeagus arises "near the posterior margin of the IXth sternite." The two statements do not agree.

I have also found that there seems to be a definite relation between the size and shape of the aedeagus and the female receptacle, as Singh-Pruthi has remarked (p. 244, par. 2). This is quite marked in some species. On p. 245, par. 2, I think he overemphasizes the intimate relations of the claspers and the aedeagus. His statement on p. 186, already referred to, I am more inclined to agree with.

Muir (1926), did not agree with Singh-Pruthi on several points. I shall give my views on some of these arguments in so far as they relate to the group studied herein. I have already discussed certain of these points in my review of Dr. Singh-Pruthi's paper.

Muir questions Singh-Pruthi's belief that the ventral portion of the pygofer is the ninth sternite (p. 325). In the Pentatomidae, and the more closely related families, at least, I am inclined to agree with Dr. Singh-Pruthi that the genital segment represents the specialized ninth sternum.

On p. 327 Muir states, "If the ventral portion of the pygofer is the ninth sternite and the genital plates new processes not represented in the other sex or in other insects, should this not be more evident in the generalized forms?" The genital plates herein described are most evident in the generalized forms of Pentatomidae. They are possibly homologous with the subgenital plates of the Homoptera. The ninth sternum is apparently always present in the Pentatomidae. The genital plates are possibly outgrowths from this sclerite.

In reference to the primitiveness of the presence of a basal plate (p. 328), this structure, with its connectives, was found in all the Pentatomidae examined. I agree with Singh-Pruthi that the ejaculatory duct passes through the basal plate, where it appears to originate, runs as a distinct duct through the "hinge", and then continues through the aedeagus proper. The sclerotization of the ejaculatory duct in the basal region is sometimes very faint, but after considerable trouble I was able to secure good mounts where the duct can be made out clearly. This is a very good excuse for overlooking it. As in the Fulgoroidea (p. 330), the claspers of the Pentatomidae function as holding organs.

V. General Description of the Male Genitalia of the Pentatomidae, Excepting Details of the Aedeagus

In the order Hemiptera the number of apparent abdominal segments varies from seven to eleven. The modes of reduction from the greater to the lesser number have already been referred to in the preceding discussion. The anterior segments of the abdomen of the Pentatomidae are usually narrower, the first sternum is usually wanting, and the spiracles are not functional caudad of the seventh segment in the adult insects. Throughout this paper the numbers given to the abdominal segments will indicate their primitive and not their apparent numerical sequence.

In the Pentatomidae no external evidence of any reproductive structures is

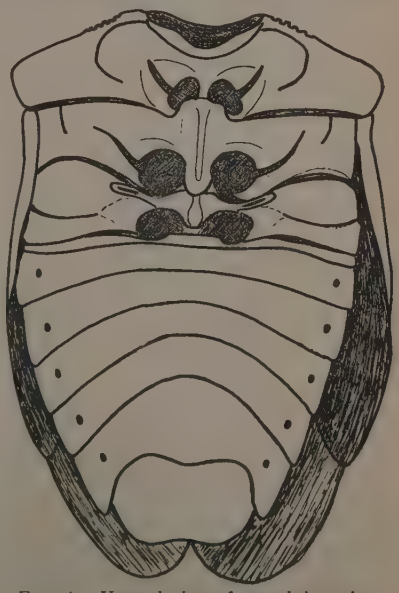

FIG. 1. *Ventral view of an adult male pentatomid.*

apparent until the final stage, that of the imago, is reached. However, in some genera a swelling of the penultimate segment of the male nymph occurs, and has been used to distinguish the sexes before maturity. This is the segment that will later contain the copulatory apparatus, or copulatoria. In general, the identity of the species and sexes in the nymphal stage are best determined by rearing. In some cases, specific characters may be safely deduced by paying special attention to the anterior regions of the body, particularly the head. The genera of the pentatomids are usually recognizable among nymphs, but the genital characters are of little or no assistance in identification until the adult stage has been reached.

In the mature male pentatomid, segments one to seven persist; there are, however, the usual changes in size, composition of cuticle, etc. (which are common to all insects), and the movement of the openings of the scent glands from the dorsal sutures of the abdomen to the sides of the metathorax. In the segments caudad of the seventh we find considerable changes taking place.

Fig. 156 is a photomicrograph, from the dorsal aspect, of the male genital apparatus of one of our commonest Canadian pentatomids, *Euschistus euschistoides*, which had previously been treated to remove all but the chitinized structures. It will be noted that several of the genital structures are lying over one another and that they, at first sight, seem somewhat difficult of homology with the genitalia of other insects. This difficulty is rather augmented by the considerable variation that occurs between many of the species.

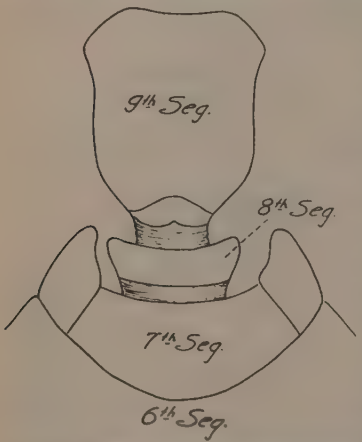

FIG. 2. *Ventral view of apex of abdomen of Euschistus sp. (male), with genital segments extended. (Somewhat diagrammatic.)*

The apparent terminal segment of the male forms a bowl or cuplike depression within which the structures directly concerned with copulation lie. The opening of this structure, herein termed the "genital-cup", may be directed dorsad (as in the specimen illustrated), directed caudad, or in many intermediate positions between these two extremes. As a general rule, the direction of the opening of the genital-cup has its counterpart in the female. Where the male opening is largely dorsad, the female structures are generally subventrad, or the female is more nearly caudad when the genital-cup of the male opens directly to the rear.

It is found that this large "genital segment" is freely movable and may be extruded from the segments cephalad to it (into which it is normally retracted to an extent that varies between species). When the genital segment is extended from the abdomen as far as it will go, the structures shown in Fig. 2 are brought into view. This drawing is of *Euschistus euschistoides* from the ventral aspect. Fig. 3 is of the same structures from the dorsal aspect, but without details

of the organs that normally lie within the genital-cup. The structure marked "8th seg.", which is brought into view only by the extrusion of the genital segment, is very apparently the remains of the eighth abdominal segment, being joined on its cephalic and caudal borders by generous folds of intersegmental membrane. This homology is confirmed by an extended study of other members of this family. At rest, in the majority of species, the eighth segment is completely retracted within the abdomen and is not usually visible externally. Miss Newell (1918) apparently overlooked this retracted eighth segment, which resulted in her naming the ninth segment the eighth.

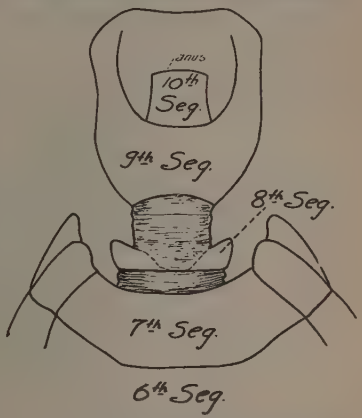

FIG. 3. *Dorsal view of apex of abdomen of Euschistus sp. (male), with genital segment extended. Details of structures within genital-cup omitted.*
(Somewhat diagrammatic.)

The Eighth Segment

This segment is generally very much reduced in size and normally retracted well within the anterior segments. In certain species, however, reduction has not progressed to the extent that it has in others (*i.e., Elasmostethus cruciatus*), and small portions of the ventro-caudal margins may be visible without the extension of the posterior segments. In all cases, with the genital segment retracted, we find the eighth segment fitting closely around the cephalic portion of the ninth segment, *i.e.*, the genital segment. It is only on the drawing out of the genital segment to its fullest extent that the relationship of the parts becomes apparent. The intersegmental membranes cephalad and caudad of the eighth segment form the main part of the connecting structure for the genital segment with the remainder of the abdomen, and permit great mobility for that latter segment. In many of the more specialized species the eighth segment appears as little more than a ring or collar surrounding this membranous terete articulation.

It is very probable that the eighth segment is largely, if not in some cases entirely, made up from the eighth sternum, with the resultant atrophy of the eighth tergum. In the retracted position the convexity of the genital segment fits closely into the concavity of the eighth segment.

The Ninth or Genital Segment

Caudad to the eighth segment is the prominent enlarged region in which occurs the depression previously referred to as the "genital-cup." This segment is homologized as the ninth, and it is the segment which contains within its depression the copulatoria. The directions assumed by the opening of the genital-cup vary greatly between groups and species. The possible evolution of the genital-cup will be dwelt on later. Where the opening of the cup is directed dorsad, the ventral portion of the segment has undergone considerable growth, and, in many cases when the genital segment is retracted within the

abdomen, the opening of the genital-cup may be almost completely hidden by the seventh tergum. Usually the wing tips cover the genitalia when at rest.

Secondary depressions occur in this genital segment in some species, particularly below the ventro-posterior margin or rim of the genital-cup. Examples of this peculiarity are seen in *Chlorochroa uhleri*, *Brochymena quadripustulata*, *B. affinis*, *Aelia americana*, etc. In other species the posterior margin of the genital-cup may be considerably produced to form a projecting liplike structure caudo-ventrad of the copulatoria, such as is found in *Euschistus* spp., *Mormidea lugens*, *Cosmopepla bimaculata*, *C. conspicillaris*, and many other species (a true hypandrium). For the purpose of uniformity, this ventral or caudal border of the genital-cup is referred to herein as the "ventral border," indicating its more primitive rather than its actual position in many species. For the same reason the margin normally above the genitalia is termed the "dorsal border," although it often might be more correctly termed the cephalic border. The adoption of this nomenclature is thought advisable, as the majority of the genital cavities open neither directly dorsad or caudad, and terms indicating the actual position of the borders in reference to the genitalia would cause unnecessary confusion and could serve no good purpose. In any case, such a system would be liable to considerable inaccuracies owing to the mobility of the genital segment.

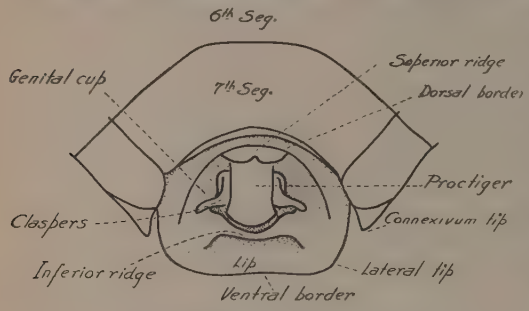

FIG. 4. *Dorsal view of the genital segment, etc., of a male pentatomid (Euschistus sp.) with the structures identified that are normally visible. (Somewhat diagrammatic.)*

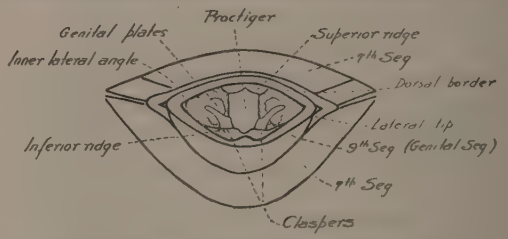

FIG. 5. *Caudal view of the genital segment of a male pentatomid with the parts identified. (Diagrammatic.)*

In many species a distinct transverse ridge may be present, cephalad to the ventral border proper (within the genital-cup). The median region of this ridge is usually closely adjacent to the tip of the proctiger. To this elevation, when present, the name of "inferior ridge" is given. Examples of this structure are to be found in *Euschistus* spp., *Perillus bioculatus*, etc.

In the more highly specialized forms, *e.g.*, *Euschistus* spp., a collarlike structure is present below the dorsal border of the genital-cup and covers the base of the proctiger. This is termed the "superior ridge" and may vary in outline.

Examples of the structures termed the "genital plates" herein can be observed readily in *Apateticus bracteatus*, etc., where they exist as quite prominent, highly sclerotized, tuberculate, platelike structures within the genital-cup and directly cephalad to the deflexed portions of the claspers. It will be observed that the edges of these structures are free on their dorsal, ventral and ental borders, but merge, without a distinct suture, into the inner surface of the genital-cup ectad. These structures occur in quite a number of the species studied.

The ventral border of the genital-cup may bear projections of many diverse forms and positions, for example: a median projection, as in *Coenus delius;* two laterally paired projections as found in *Banasa dimidiata;* a single pair as in *Acrosternum hilaris*, placed well apart; paired projections close to the median line, as in *Podisus maculiventris;* and a host of other modifications of outline and structure of the dorsal, lateral and ventral margins, which makes the study of the morphology of the genital segment a very fertile field for the systematist and the phylogenist.

The Proctiger

Lying within, and attached to the dorsal inner surface of, the genital-cup is a flaplike structure which is movable dorso-ventrally, but is not capable of any very considerable lateral movement. At its distal extremity the anus is situated. The position of this structure is indicated by "10th seg." in Fig. 3 of *Euschistus euschistoides*. It invariably occupies a position dorsad to caudad to the aedeagus, covering and protecting that organ *in situ*. Whether the position be dorsal, dorso-caudal, or caudal depends on the direction of the opening of the genital-cup. Sharp's term of rectal-cauda, while very descriptive, is replaced by that of proctiger, and this structure is herein homologized as the tenth, and possibly the remains of the eleventh, segment of the primitive abdomen.

The upper surface of the proctiger is always more highly sclerotized and ornamented than the under surface. In the majority of the forms examined the apex of this structure is bent downwards, so that the anal opening rests against the posterior (or ventral) inner surface of the genital-cup. In this position the tip and under surface would not be visible externally and, as might reasonably be expected, would not be so highly sclerotized as the upper surface (which is the exposed portion). In some species the proctiger rests in an almost vertical position caudad to the aedeagus. In all species it is cernuous. In this paper the descriptive notes on the proctiger of the different species refer to the upper surfaces only.

In transverse section, when at rest, the proctiger is found to form a more or less vaulted or arched structure, being convex without and concave on the under surface. The superficies, being the more highly sclerotized portion, does not permit of very much change in form, but the membranous, less highly sclerotized under surface does. The result is that the under surface of this structure tends to conform to the morphology of the upper surface. In consequence, when the proctiger is not expanded it forms a curved covering for the aedeagus, which lies beneath it within the genital cup.

In some species, transverse indentations on the dorsal side of the proctiger permit limited lateral movements of the structure and facilitate the normal dorso-ventral movements, both of which movements are best compared to those executed by the abdomen of a crayfish. Except in a few genera, very little or no bending of the organ dorsad, further than straightening the natural longitudinal convexity, is possible. Dorsal straightening and ventral flexion are two general movements which are possible in several species. A third and a fourth movement of the proctiger will be described in my notes on the act of copulation.

During feculation, a membranous structure is often extruded from the apparent anal opening. Normally these folds of membrane have been found to lie invaginated within the chitinized walls of the proctiger proper. These folds of membranous tissue serve the purpose of conveying the fecula beyond the limits of the genital-cup. Sharp (1890) was somewhat puzzled to know how this act was accomplished. The true anal opening is really situated at the posterior extremity of this accessory structure, and it is visible only on evagination of these adjacent membranes. The possibility of these folds of membrane representing rudiments of the eleventh abdominal segment is discussed later. Pressure on the abdomen of a fresh adult specimen is often sufficient to bring these structures into view, usually resulting in the elevation of the tip of the proctiger from the floor of the genital-cup and the evagination of the parts discussed. The true anal opening can usually be distinguished by the presence of fine rows of hairs which often surround it, and by the slight thickening of tissues immediately adjacent to it.

It is doubtful if any muscles are directly employed in raising the proctiger during feculation, and it seems more probable that the pressure of the fecula within the structure would cause the less highly sclerotized ventral wall to become externally convex, which, pressing on the structures below, would cause the elevation of the more or less rigid dorsal wall and the evagination of the anal membrane.

The Claspers

Within the genital-cup, and situated on either side of the proctiger, are the clasping organs, or "claspers." These structures have their origin from the posterior margin of what I consider is the ninth abdominal segment. The term clasper, while descriptive as far as function is concerned, is used rather generally in other groups of insects to designate other structures as well as those arising from the ninth segment.

The claspers vary so widely in shape that few general statements can very well be made. Usually there is a more or less abrupt lateral outward curving from where they arise on either side of the aedeagus and project beyond the sides of the proctiger. They may be flattened posteriorly on their external surfaces, smooth on their inner surfaces, bear one or more projections, be widely branched, pronglike in structure, minute or large. Their relative position on either side and slightly beneath the aedeagus is, however, very constant.

In all species examined, the claspers were found to be usually very hard sclerotized organs. In *Zicrona caerulea* the organs are more exceptionally slender and delicate. In general, the claspers may be said to be capable of lateral movement only; that is to say, in those insects where the genital-cup opens squarely to the rear, the movement of the tips of the claspers describes an arc caudad from their lateral position to that of the median line. In such species as *Euschistus* spp., etc., where the opening of the genital-cup has proceeded dorsad, the movement of the claspers is the same in relation to the opening of the cup but has necessarily changed in relation to the remainder of the insect's body.

As will be explained more fully later, the formation of the genital-cup in the ninth segment results in a posterior chamber which is open externally, *i.e.*, the genital-cup, and an anterior chamber which is open internally only, *i.e.*, the remaining anterior portion of the original cavity within the primitive segment. The dividing wall between these two chambers is the wall to which Sharp (1890) has given the name "diaphragm", but without, I feel sure, realizing its possible origin. The term, however, is descriptive and will therefore be used.

The claspers penetrate this diaphragm, referred to above, and continue well into the anterior chamber of this ninth or genital segment. These apodemes of the claspers often give off specific processes for the attachment of muscles. The apodemes do not pass forward of the ninth segment, and the movement of the claspers appears to be controlled entirely by muscles within this segment. There is no articulation between the external and internal portions of the claspers; movement of the internal portions would therefore necessarily result in movement of the external portions.

The very considerable differences in form that exist between the claspers of species, and their accessible nature, make them very important structures to the taxonomist.

VI. The Aedeagus and Claspers of the Pentatomidae, and Their Connections

In male pterygote insects the conducting tubes from the reproductive organs usually open through a single genital aperture, which is located in the membrane connecting the ninth and tenth abdominal sterna (slight migration from this region appears to have occurred in certain groups of insects, so that the opening is apparently located in the ninth sternum itself).

From the genital aperture there is usually formed, through invagination of the body wall, a slender conducting tube, the ejaculatory duct, which joins up with the two primitive conducting tubes, the vasa deferentia. The invaginated portions of the seminal passages, then, would be distinguishable from those of internal origin by their chitinization. In Ephemerida and Dermaptera this invagination does not take place, and two apertures are sometimes present. Where one only is to be found, it is presumed that one of the openings has atrophied or the two openings of the ducts have fused.

The genital apertures, whether paired or single, are usually borne on an outgrowth, the penis or aedeagus. Where there are two apertures, there are two penes. In the great majority of insects there is just one aperture and correspondingly but one outgrowth, whose walls are often more or less sclerotized. The occurrence of a double penis is probably a very primitive condition, and the penis or aedeagus may very possibly have developed independently in certain orders.

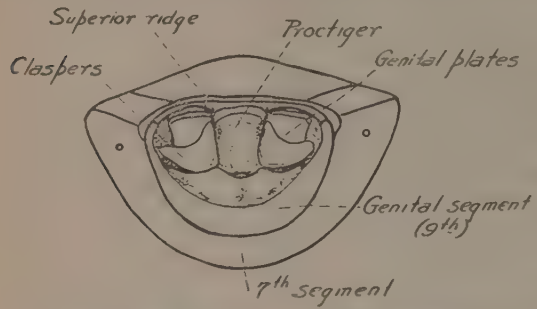

FIG. 6. *Caudal view of the genital segment of Perillus bioculatus with the structures in situ.*

In the Pentatomidae, the aedeagus is not visible when the genital structures are at rest. It is not until the proctiger is raised or withdrawn that the aedeagus is brought into view, as it lies beneath (or behind) the proctiger, which serves as a protective covering for it. The aedeagus is attached to the deepest region of the genital-cup, beneath the point of junction between the proctiger and the genital segment (ninth). If the homologies of the adjacent sclerites are correct, this means that the aedeagus extends into the genital-cup from between the ninth and tenth sterna of the abdomen. This position is in harmony with that rather generally agreed to be the primitive position of this organ in the majority of insects. On either side of the aedeagus are the claspers. The relative position of the aedeagus is shown in Fig. 6 and 7, which represent caudal views of the genital structures of *Perillus bioculatus* Fabr. In Fig. 7 the proctiger has been removed in order that the position of the aedeagus could be illustrated.

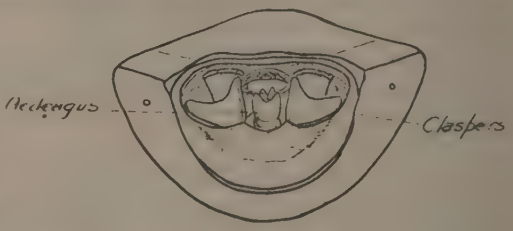

FIG. 7. *Caudal view of the genital segment of Perillus bioculatus with the proctiger removed to show the relative position of the aedeagus.*

A study of the details of the structure of the aedeagus in the Pentatomidae revealed some very startling differences between genera and species. The extreme delicacy of some of the structures rendered it difficult to prepare good mounts. To figure the aedeagus it is necessary to mutilate the insect specimen. This fact renders the aedeagus of less practical importance as an aid to classification than are the more available adjacent structures.

A few of our common and representative species, of which I possessed a plentiful supply of fresh specimens, were selected for study. These included

several species of *Euschistus*, particularly *E. euschistoides* and *E. tristigmus*, *Coenus delius*, *Cosmopepla bimaculata*, *Perillus bioculatus*, *Podisus maculiventris*, etc. All these species exhibited very considerable differences.

Fig. 158 and 157 are photomicrographs of the aedeagus of *Euschistus euschistoides* and *E. tristigmus*. Fig. 8 represents a line drawing of the aedeagus of *Euschistus euschistoides* from the dorsal aspect. In many details this figure is rather more diagrammatic than actual. In this genera, as in some others, the aedeagus gives strong superficial evidences of asymmetry. The basal region of the aedeagus is attached in the genital-cup beneath the proctiger. In Fig. 160 the aedeagus of *Euschistus euschistoides* is photographed within the genital-cup, with the proctiger removed. It will be noted that the basal portion of the aedeagus appears to be telescoped within itself. The basal accommodation allows for the necessary movements of the aedeagus during copulation.

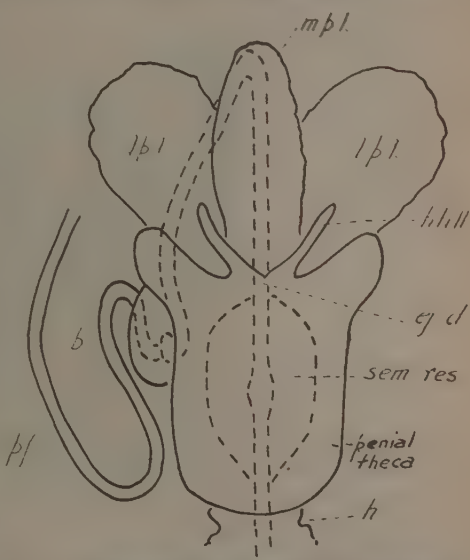

FIG. 8. *Dorsal view of the aedeagus of Euschistus euschistoides. l.p.l. lateral penis lobe; m.p.l. median penis lobe; b. bulb; p.f. penisfilum; ej.d. ejaculatory duct; sem. res. seminal reservoir; h. hinge; titill. titillators.*

It is the major region of articulation and, as will be shown later, permits the extension of the aedeagus from within the limits of the genital-cup. Distad to the hinge is that part to which Sharp (1890) gave the descriptive term of "theca", but very evidently without being able to explain the true relation of the adjacent parts. This part is herein termed the penial theca. The penial theca terminates, in this species, in two rounded projections. There is no indication of any definite division between the penial theca and these projections, the latter being but distal extensions of the former.

Fig. 9 is a line drawing showing the lateral aspect of the aedeagus of *Euschistus euschistoides*. Fig. 158 is a photomicrograph (panchromatic plate) of the same organ viewed from the same aspect. From the "hinge" the ejaculatory duct may be seen passing distad into the interior of the more highly sclerotized penial theca. Midway within the penial theca it is joined by another duct, of approximately equal size, which runs parallel to it basad. This secondary duct, or ductus seminalis, curves dorsad and then distad within and, near the basal region of the penial theca, enlarges into a conspicuous "seminal reservoir," which occupies the most of the region within the penial theca above the ejaculatory duct proper.

From the dorso-distal region of the penial theca arise two conspicuous palpuslike structures. They are unsegmented. It is very probable that these function as titillators and may assist in holding the aedeagus within the genital organs of the female. Walker (1919), in his work on the terminal structures of Orthopteroid insects, was inclined to view the titillators of most Orthoptera as parameres, which he describes as being "primitively, a pair of chitinous processes, arising near the base of the penis or from its walls, and having typically a lateral or dorsal-lateral position." The homology of the structures herein termed the "titillators" is uncertain, but they are possibly secondary modifications.

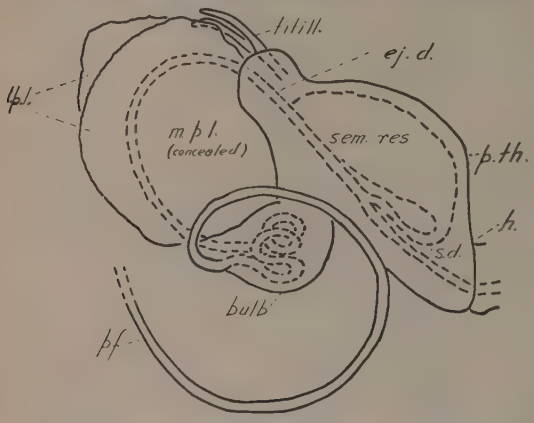

FIG. 9. *Dextro-lateral view of the aedeagus of Euschistus euschistoides. p.th. penial theca.*

From the distal borders of the penial theca there arise three thinly sclerotized saclike structures, which appear to have no definite fixed outline. These structures may lie normally slightly within the penial theca, but during copulation are prominently everted and distended. To the lateral sacs of this organ, the penis, I have ascribed the term "lateral lobes of the penis." The median structure is herein termed the "median lobe."

From the point of union of the ejaculatory duct and the seminal duct a single conducting tube (the continuation of the ejaculatory duct), runs distad through the penial theca, beneath and closely adjacent to the seminal reservoir, and into the median lobe of the penis proper. Within the median penis lobe it curves evenly ventrad to emerge within a bulblike structure dextrad and below the penial theca and penis lobes. This "bulb" is continuous with the median penis lobe; it is not fused to the adjacent penial theca and lateral penis lobes, although it rests in close contact with them. Within the "bulb" the ejaculatory duct winds dorsad twice and then ventrad once, to emerge distad, and immediately curve strongly basad. To this free portion of the ejaculatory duct the term "penisfilum" is given. In other species, where it projects as a slight spine, the term "virga" might be better employed. Even in the latter case, however, it is often capable of extension. The penisfilum of *E. euschistoides* winds itself, like a coiled watch spring, into three complete loops, the termination coming to lie along the median line of the distal region of the median penis lobe. Dissection of the penisfilum was attempted, and the general impression was obtained that it is formed through the fusion of two lateral pieces. Thus, while the aedeagus is resting in a position that is not

symmetrical, the actual development of parts has been much more symmetrical than a cursory examination would indicate.

The aedeagus of *Coenus delius* (Fig. 10 and 159) is easy to homologize with that of the foregoing, and very plainly represents a more primitive, and possibly an intermediate stage in the specialization that has been obtained in *Euschistus* spp. All of the essential structures described above, with the exception of the "bulb", are present. Projections distad of the penial theca are indistinct, but the titillators are more prominent than in *Euschistus*. The penis is composed of three lobes (as in the foregoing) but the ejaculatory duct extends but little distad into the median penis lobe and curves almost immediately ventrad to emerge as the penisfilum. The latter curves sharply basad and then dorsad, to emerge from between the median and left lateral penis lobes near the dorsal region of the point where the lobes join the penial theca. From this position the penisfilum curves basad and ventro-distad to form a single incomplete circle. As in the case of *Euschistus*, the penisfilum normally lies to the right of the aedeagus (*i.e.*, on the right side in reference to the remainder of the insect's body). In neither of these examples is there any sign of specialization of the tip of the penisfilum.

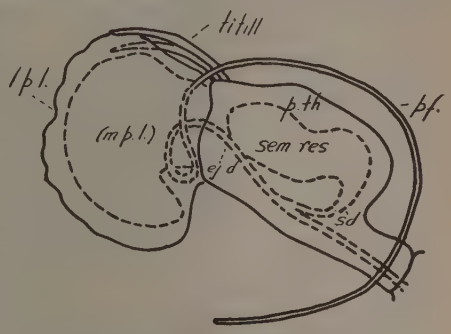

FIG. 10. *Dextro-lateral view of the aedeagus of Coenus delius Say.*

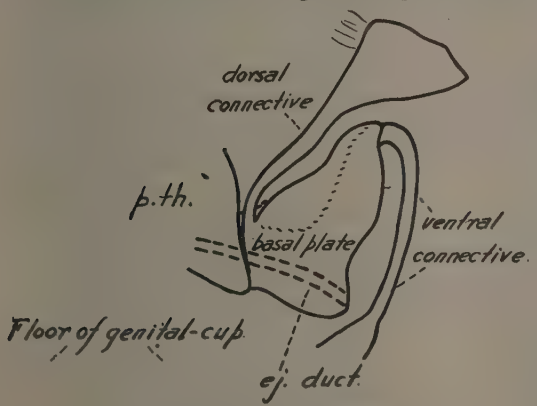

FIG. 11. *Lateral view of structures at base of aedeagus of Euschistus tristigmus.*

The basal attachments of the aedeagus of *Euschistus tristigmus* are shown in Fig. 11. This represents a lateral view of these structures. The "basal plate" is the most prominent and easily noted structure. The less thickly sclerotized region between the penial theca and the basal plate, which is a region of articulation, is termed the "hinge". The relation of the other parts to the basal plate is clearly shown in the figure. In this position the aedeagus is in an intermediate position, being neither fully extended nor retracted.

From the caudo-dorsal sides of the basal plate arise two sclerotic arms, which are herein termed the "dorsal connectives". These run cephalo-dorsad

from the basal plate. When the aedeagus is extended these dorsal connectives have a lifting and restraining function. From the disto-lateral regions of these connectives there are usually evidences of slight chitinous threads running to the central region of the claspers. These are not always well marked. The distal portions of these connectives have muscular attachments which run cephalad into the genital segment.

The "ventral connectives" of the basal plate are also shown in the figure referred to above. These sclerotic structures arise from the cephalo-dorso-lateral regions of the basal plate. They run ventrad and caudad to merge into the floor of the genital-cup. This means that the basal structures of the aedeagus are directly continuous with what I consider is the primitive ventro-caudal border of the ninth sternum.

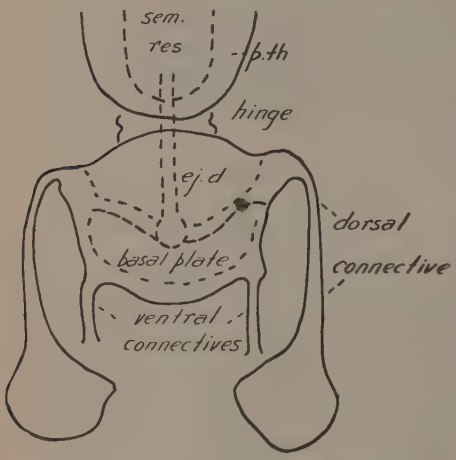

FIG 12. *Base of aedeagus of Euschistus tristigmus. Dorsal view. Basal plate of aedeagus drawn proximad. Position when aedeagus is extended.*

Fig. 12 shows a dorsal view of the same structures as those described above, as they appear when the aedeagus is extended. The basal plate has moved distad until checked by the ventral connectives. A further restricting action is exerted by the dorsal connectives, which also tend to lift the distal edge of the basal plate. At the same time the ventral connectives have pulled the proximal edge of the plate ventrad. The net result is that the aedeagus, as it is extended, tends to point more dorsad. I know of no object to which it is possible to compare the basal structures and their movements. Perhaps the action can be better understood by stating that, with the extension of the aedeagus, the ventral region of the basal plate moves caudo-dorsad. On retraction, the reverse action occurs (see Fig. 13). The somewhat triangular shape of the basal plate, when viewed laterad, must be borne in mind.

Fig. 14 represents the aedeagus of *Euschistus tristigmus* as it looks when its tip is pressed back until it points cephalad. In this figure the relationships of

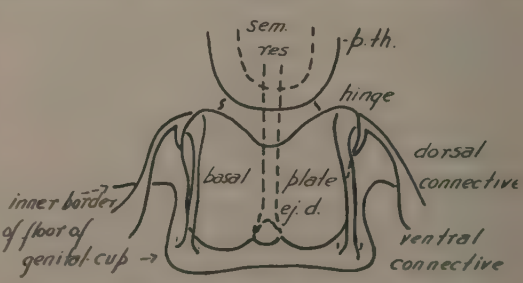

FIG. 13. *Dorsal view of base of aedeagus of Euschistus tristigmus. Basal plate of aedeagus drawn distad. Position when aedeagus is not extended, except that aedeagus is drawn into the basal plate concavity more closely than is shown.*

the ventral connectives to the basal plate and the floor of the genital-cup are shown as clearly as possible. In the last four figures mentioned, the position of the ejaculatory duct at the base of the aedeagus can be noted.

The basal plate of *Apateticus bracteatus* is, superficially, a comparatively simple affair. The general, somewhat dustpan-like shape, can be noted from Fig. 15. The relative positions of the ventral and dorsal connectives are the same as those previously discussed. This drawing has been made with the basal plate turned slightly to one side in order to show the depth of the sides formed by the lateral sclerotizations.

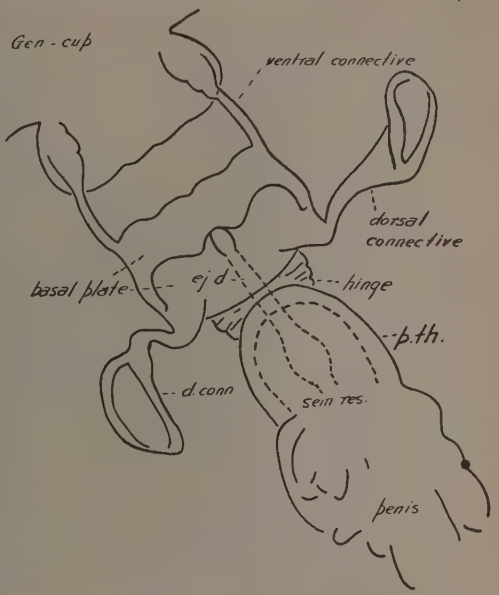

FIG. 14. *Aedeagus of Euschistus tristigmus. Aedeagus pressed backward into anterior chamber of genital segment in order to show the ventral surface and the basal connections.*

Fig. 16 shows the aedeagus of *Cosmopepla bimaculata*. Most of the structures are essentially the same as those already described. The hinge is present and serves as the articulating structure between the aedeagus proper and the basal plate. The penial theca is very apparently the more highly sclerotized region distad to the hinge and enclosing the seminal reservoir. The relations of the seminal reservoir, seminal duct, and the ejaculatory duct, within the penial theca, are essentially the same as those previously described. Lateral lobes of the penial theca and the titillators are absent in this species. From the distal borders of the penial theca arise a pair of thinly sclerotized sacs, the lateral penis lobes, which are capable of being withdrawn within the penial theca to a considerable degree and very strongly everted during copulation. The medium penis lobe

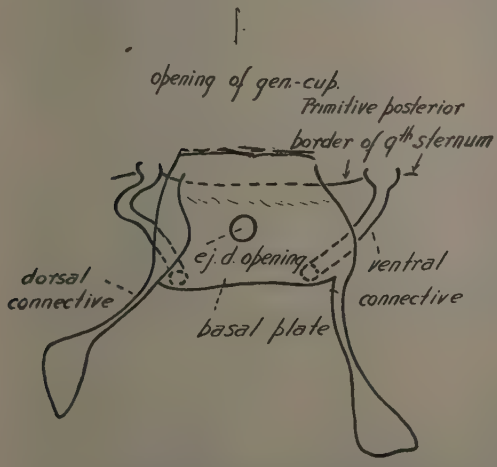

FIG. 15. *Dorsal view of the basal plate and connectives of Apateticus bracteatus. (Basal plate tilted to one side to show depth better.)*

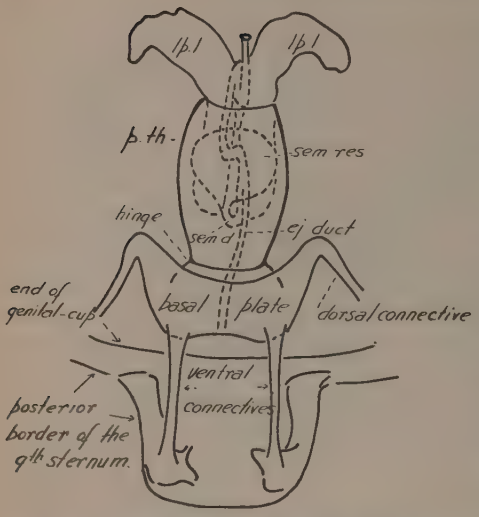

FIG. 16. *Dorsal aspect of the aedeagus and its basal connections of Cosmopepla bimaculata. Aedeagus extended from genital segment.*

is absent, and the ejaculatory duct curves downward, then upward and distad, to end in a slightly enlarged spinelike projection between the two penis lobes. The basal plate, hinge, ventral and dorsal connectives and their attachments are, in their essentials, the same as the preceding species. The relations of the ventral connectives to the infolded posterior border of the ninth sternum are even more marked here, as this invagination of the genital segment has not progressed to the same extent as in the case of *Euschistus* spp. and *Cosmopepla*.

Fig. 17, showing the aedeagus and basal connections of *Perillus bioculatus*, presents a form that is somewhat less closely allied to the preceding species. The penial theca of the aedeagus is quite well marked and encloses the usual large seminal reservoir, etc. Unlike the foregoing species, the penial theca extends distad to form a cup-like structure around the penis. Paired eversible sacs are present, as in the other species described, and are undoubtedly the lateral penis lobes. A median lobe of the penis, which is cleft medianly at its tip and with the end of the ejaculatory duct projecting slightly from the base of this cleft, lies between the two lateral penis lobes. This median lobe is more heavily sclerotized than are the lateral lobes. From the looped appearance of the ejaculatory duct in the median lobe of the penis it is thought that either the ejaculatory duct itself is capable of extension from the median lobe or that the median lobe moves from within the protecting distal extensions of the penial theca during copulation.

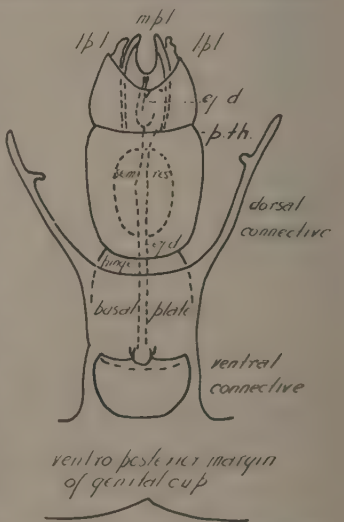

FIG. 17. *Aedeagus and basal connections of Perillus bioculatus. Aedeagus directed cephalad. Drawing from dorsal side of insect but ventral view of the aedeagus itself.*

Fig. 18 shows the aedeagal structures of *Chlorochroa uhleri*. Most of the parts can be easily homologized with those already figured. As in the case of *Perillus bioculatus*, the median lobe of the penis is cleft, more heavily sclero-

tized than the lateral lobes and, in addition, is asymmetrical. In this case, the ejaculatory duct projects strongly from this median cleft of the median lobe. The lateral penis lobes are of a different shape from those previously described herein, having a thickened distal fringe and not being of the same shape. The penial theca and the contained seminal reservoir are quite large and conspicuous. The basal structures are more complicated than those found in the predacious species. Both the dorsal and ventral connectives are identified, but these are supplemented by other structures arising from the proximal region of the basal plate. This species, both in the form of its genital-cup, etc., as well as its aedeagal structures, indicates a rather independent line of development.

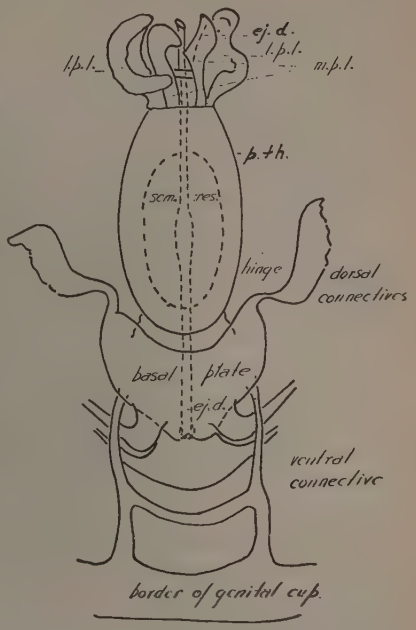

FIG. 18. *Aedeagus and connectives of Chlorochroa uhleri. Aedeagus bent backward (cephalad) to expose its ventral surface. Drawing from dorsal side of insect.*

The ventral connectives of the basal plates of all species examined are firmly fused with the inner margin of the floor of the genital-cup. If sufficient energy is exerted to separate these parts, the basal plate will tend to break apart from the ventral connectives, not the connectives from the genital segment. Both the attachments of the ventral connectives are quite firm and do not come apart very easily. The aedeagus is intimately connected with its basal plate. This means, according to the views of the writer, that the aedeagus and all its parts are practically continuous with the ventro-caudal margin of the ninth segment. Fig. 19 is a dorsal view of the interior of the genital segment of *Euschistus tristigmus*, drawn to show the relation of the ventral connectives of the aedeagus to the genital segment, etc.

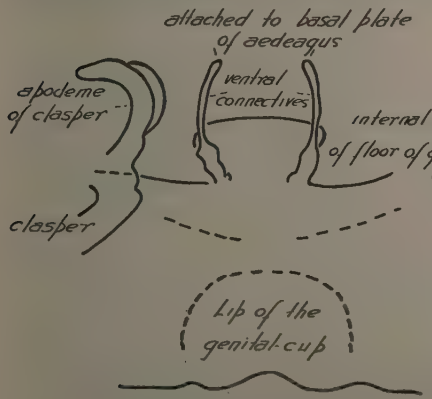

FIG. 19. *Dorsal view of the interior of the genital segment of Euschistus tristigmus to show attachment of aedeagus, etc.*

The two claspers lie on either side of the aedeagus. From the work of other authors, one might incline to the impression that these structures are very

intimately connected with the aedeagus. This is not the case. The connection with the aedeagus exists, but it is by no means as well marked as the independent connection the claspers themselves possess with the segment.

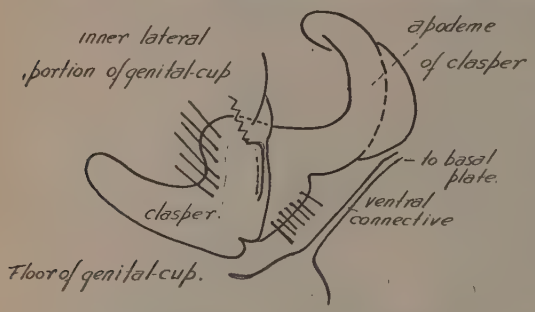

FIG. 20. *Dextral view of sinistral clasper and attachment of Euschistus tristigmus. (Ental portion of clasper pressed ventrad somewhat to show the shape, etc., better.)*

The clasper is a solid structure, usually quite heavily sclerotized. Only about half, or less, of its total length is visible. The non-visible portion extends as an apodome well into the anterior region of the genital segment, where it furnishes points of attachment for muscles which control the movements of the clasper. The action is that of a lever, the fulcrum being at the point where the clasper emerges from the interior of the genital segment. Movement of the apodeme laterad would result in the free and visible end of the clasper moving entad, and *vice versa*.

The inner lateral walls of the genital segment curve up closely on either side of the claspers. From the inner free edge of these walls there is a distinct continuous connection between clasper and the genital segment. These connectives run to the dorso-ental portion of the clasper in the region of its fulcrum. Both ends of these connectives are fused to the parts they join, and the connections are quite distinct. Fig. 20, which is a dextral view of the sinistral clasper and its attachments of *Euschistus tristigmus*, shows these features quite clearly. There is no connection between the ventral connectives of the basal plate and the clasper or clasper connectives. The relation of these latter parts to each other is shown diagrammatically in Fig. 21. Any connection that exists between claspers and the aedeagus is by way of the latero-distal extremities of the dorsal connectives of the aedeagal basal plate. This connection is by no means as well marked as those already described.

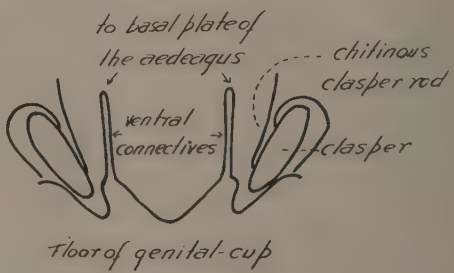

FIG. 21. *Caudal view of the interior of the genital segment of Euschistus tristigmus to show attachment of claspers and the relation of the aedeagal connectives. (Somewhat diagrammatic.)*

It its essentials, then, the whole aedeagal structure consists of two connectives arising from the primitive ventro-caudal margin of the ninth segment, which run to a relatively large basal plate, with its four thicker sclerotized regions to which the aedeagus proper and a pair of dorsal connectives are attached. Between the ventral connectives the infolded margin of the ninth

sternum often extends further cephalad than at the sides. The aedeagus proper consists of a basal articulation, termed the hinge, which continues as a more heavily sclerotized cylindrical structure, the penial theca, and encloses the large seminal reservoir, which arises from the ejaculatory duct. At the distal end of the penial theca the more thinly sclerotized penis lobes are situated. Of these there may be two or three. The shape and size of the median lobe vary; to a lesser extent, this is true of the lateral lobes as well. The ejaculatory duct appears to pass through an opening in the small median sclerotization of the basal plate. Its continuation was not noted cephalad of this point. The duct is distinct from the basal plate to its distal termination. being joined where it passes through the penial theca by a duct from the seminal reservoir. The ejaculatory duct passes into the median penis lobe, when one is present. In the absence of a median lobe the duct may continue as a free structure for a short distance from between the two lateral lobes. Considerable specialization of the ejaculatory duct may occur, and in some species a very long conspicuous penisfilum is formed. In such cases the symmetry of the parts is temporarily lost, as the coiled penisfilum comes to lie on one side (dextral) of the aedeagus and carries with it adjacent portions of the median penis lobe. This section of the lobe may become specialized on that side to accommodate the internal commensurate coiling of the penisfilum which allows its ready extension. This region has been already mentioned as the bulb. Secondary structures and other specializations have been referred to.

VII. The Act of Copulation in the Pentatomidae

While details of the act of copulation among different species of the Pentatomidae would require considerably wider observations than those made, a sufficient number of species have been observed to justify a few general statements. These statements are based on records of the activities of many caged specimens and observations on the act of copulation made in the field during collecting trips.

As far as could be ascertained, there is nothing approaching "courtship" in insects of this group. The female, as a rule, shows complete indifference to the male at all times, seeking to avoid rather than facilitate the advances of the members of the other sex. Olsen states that the male, "shows signs of great affection." I failed to observe this. Male and female meet and employ their antennae actively over each other's body, but this was observed to also occur when insects of the same sex met. It appears evident, however, that the sexes are able to recognize each other by this contact as two males meeting in this manner would soon evince no further interest in each other.

The natural position for the insects to assume in coitu would be posterior to posterior, *i.e.*, facing in opposite directions with the caudal extremities of their abdomens in contact. In this position the genital organs would be in their right relation to one another for the act of copulation. In practice this position is usually the final, but seldom the initial position assumed.

The female, as has been pointed out, shows almost complete lack of any interest in the male. The male, however, eventually manages to mount the back of the female. This is accomplished by stealth, or by sudden onslaught. On mounting, the position is usually head to head, and the male moves sidewise until its venter is ventro-laterad to the body of the female. The above movements are usually executed quite rapidly, with more or less strenuous attempts on the part of the female to dislodge the male. When this sidewise movement has been successfully accomplished the genital segment is evaginated from the male's abdomen, exposing the rudimentary eighth abdominal segment and the intersegmental membranes. The extended genital segment appears to be under perfect muscular control and is capable of remarkable manipulation. Movement is possible in all directions, even to complete turning over of the genital segment. As the correct and necessary relation of the genital parts between male and female is posterior to posterior, the protruding genital segment must obviously be capable of considerable mobility in order to arrive at this relation from the initial position described. The indicated position is what eventually occurs.

With the act of union accomplished, the insects may remain in the initial position for some time, or the male may release its hold of the anterior part of the body of the female and take up a position to the rear. When the latter position is adopted, the insects may remain motionless for some hours, a forward movement of one necessitating the backward progression of the other.

It was frequently noticed that the insects could be found in coitu a great deal when feeding, and the male was observed as often taking advantage of the insertion of the beak of the female, as it necessarily tended to restrict her movements. Copulation did not appear to interfere with the feeding activities of the female, and it was a very common sight to see the females feeding while in coitu.

The length of time these insects remain connected appears to vary a great deal. While no definite records were kept of this feature, it was observed that some did not remain together longer than twenty minutes, while others remained in this relation the better part of an afternoon.

The species that were observed in captivity in any number were *Podisus maculiventris*, *Chlorochroa uhleri*, *Euschistus euschistoides*, *Podisus serieventris*, and *Coenus delius*.

Mention has been made by some authors of the males crawling under the bodies of the females, so that the dorsum of the male becomes adjacent to the venter of the female. This position, while possible, I do not believe is general, nor is that of an initial posterior to posterior position. While both these relations may very possibly occur, the method outlined herein appears to be the more common procedure.

VIII. The Working of the Genital Structures

The position and general structure of the aedeagus in the Pentatomidae have already been discussed. Lying, as it does, beneath the overlapping proctiger,

and usually deeply within the genital-cup, it is at first rather difficult to understand how union is effected with the female. The proctiger is capable of but slight lateral shifting, and the aedeagus does not normally project beyond the borders of the genital-cup.

Working with freshly killed specimens of *Perillus bioculatus*, it was found that inward pressure applied to the proctiger caused the tips of the claspers to come together. Insects, of the same species, were etherized and observed under the binocular as they came from under the influence of the anaesthetic. It was found that the mouth-parts, and the genital structures, were often rendered active, the stylets of the mouth being thrust in and out and movements of the genitalia occurring. It was observed that the movements of the claspers were entirely lateral, the tips swinging together to meet on the median line caudad to the proctiger. When the clasper tips come together, the proctiger is almost hidden except for its median ridge, and is pressed or retracted cephalad. On the claspers moving laterad, the proctiger moves caudad again to its normal position, its tip elevates dorsad, and the expanded tip of the aedeagus is thrust from underneath. Examination of live specimens of several other species revealed the same phenomena, but with additional modifications according to the degree of specialization of structure. In *Podisus maculiventris* the working of the structures is quite similar to that described.

The functions of the claspers, then, appear to be twofold. When the tips swing together they form a wedgelike structure, with which the male effects an entrance between the sclerites of the genitalia of the female. This function is general, I believe, throughout the family. On effecting an entrance into the female the claspers must necessarily spread apart, back to their normal position, in order to allow for the erection of the aedeagus. In this position they undoubtedly act as clasping organs to hold the female until the act of copulation is completed.

In several species, *e.g.*, *Euschistus* spp., it will be observed that the exposed surface of the proctiger is not of uniform composition throughout its length, the basal half being smooth, shining, and with numerous transverse sutures. In such cases it will also be noted that the union of the proctiger is obscured beneath the "superior ridge." In such species, the proctiger is capable of being retracted within the anterior chamber of the genital segment until only part of the differentiated area on its distal region remains visible. This movement results in the partial uncovering of the aedeagus, and allows the latter freedom of movement that would make it otherwise almost impossible to understand how the act of union between male and female could be accomplished.

On the proctiger of *Peribalus limbolarius* occurs a median inverted V-shaped area, which extends from the tip about four-fifths of the way basad. This area is a diagonally striated invagination, which, being less highly sclerotized than the remainder of the exposed surface, permits dorsal flexion of the proctiger. A similar specialization occurs in *Trichopepla semivitatta*, and to a lesser extent in *Trichopepla atricornis*. Normally, in these species, the tip of the proctiger is somewhat dorsally deflexed, but it seems reasonable to suppose that this is

another method by which the necessary freedom of movement of the aedeagus during copulation is allowed for.

It was thought possible, at first, that the claspers might be concerned in the emission of the semen by means of lateral pressure being brought to bear by them on the base of the aedeagus. On closer study it was found that there is normally quite a gap between the aedeagus and these organs. With the intervention of the lateral borders of the proctiger the necessary pressure might be obtained, but during copulation this organ would not be adjacent to the aedeagus, and the latter caudad to the claspers. If any pressure by the claspers is obtained, it seems probable that it can only be brought to bear on the hinge of the aedeagus, and what purpose this would serve is problematical. Further, the claspers are spread apart after entrance into the female has been accomplished.

Speculation has arisen as to how the aedeagus is capable of effecting the necessary union with the female organs. Sharp was not able to offer any explanation. In some of what I consider are the more highly specialized forms within this group, the position of the aedeagus is so deeply situated within the genital-cup, as well as being beneath the proctiger, that such speculation would appear to be well justified. The difficulty arising from the position of the proctiger I have already discussed, but even with this obstruction removed, union between the sexes in many of our species would still be impossible if the aedeagus itself were not capable of some movement from within the genital-cup. Sharp (1890) did not consider that the aedeagus was capable of being extended to any extent, and yet was unable to explain satisfactorily how copulation could be accomplished otherwise. Fig. 160 is a photomicrograph of the aedeagus of *Euschistus euschistoides* lying within the genital-cup, and with the proctiger removed. The aedeagus is lying slightly on its side. The point to which attention is drawn is its apparent invaginated or telescoped position. Fig. 161 is a photomicrograph of the same structure of the same insect, from which the proctiger has also been removed, but with the aedeagus extended from within the genital-cup. If the relative positions of the aedeagus and the claspers be noted in these two illustrations, the degree of extension may be judged better, and also the relation of the claspers to the sides of the aedeagus both normally and when the aedeagus is extended. If these illustrations be compared with that of Fig. 156, which represents the complete structures in their normal position (including the proctiger), the foregoing features are still further emphasized. It will be observed that the claspers do not move forward with the aedeagus.

In the study of the aedeagus of those species of Pentatomidae which possessed a penisfilum, a cursory study of the female structures in the same species was made in order to see what accommodating structures might exist for this male organ. While it is unfortunately beyond the limits of this paper to discuss these findings in detail, it may be stated, that, so far as could be ascertained, where a penisfilum exists there is an accommodating tubelike structure in the female. Usually it appears to be of equal length to the penisfilum of the male of the particular species, and, I presume, for the reception of that organ

during copulation. It exists on the opposite side of the body to that of the penisfilum of the male. This means that when the normal position of posterior to posterior of the genital organs of male and female is assumed during copulation, the two structures referred to would be directly caudad to each other. Fig. 162 is a photomicrograph showing a dorsal view of the internal genital structures of the female as exposed when the terga of the abdomen are removed. All the revealed structures are chitinized and the soft musculature, etc., is not shown.

The lateral penis lobes are usually strongly distended during copulation. In those forms where these lobes are normally partially inverted within the penial theca it is very probable that eversion results here also.

Among those species possessing a penisfilum it will be noted that the coiling of this organ, when viewed dextrad, is invariably clockwise. Within the anterior of the "bulb", where one exists, or of the median penis lobe when a bulb is absent, the coiling of the ejaculatory duct is always anti-clockwise. On the aedeagus of *Euschistus euschistoides*, it will be seen that the penisfilum executes three complete turns. In the same insect the ejaculatory duct coils in the opposite direction the same number of times. The penisfilum of the aedeagus of *Coenus delius* executes but one complete turn. Within the median penis lobe the ejaculatory duct of this latter species turns but once in its anti-clockwise direction. From the above it would appear very improbable that any uncoiling of the penisfilum is necessary, but that the straightening of the penisfilum caudad would result in a straightening movement of the terminal internal portion of the ejaculatory duct within the bulb or the median penis lobe.

The movements of the basal plate of the aedeagus and the connectives during copulation have already been referred to in the description of those parts.

(*To be concluded in next issue*)